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LOCAL IMMUNITY IN ROTAVIRAL INFECTIONS

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Abstract

After a short introduction into the mechanisms of nonspecific defence in the intestine, a brief review on specific, local intestinal immunity is given. Results of experiments on local and humoral antibody production carried out in five caesarian-derived, colostrum-deprived calves are presented. In each of the calves, two jejunal fistulas were prepared, and samples from fistulas and faeces were taken for antigen and antibody determinations at intervals for up to 120 days post infection. Antibodies in the jejunum appeared between 3 and 12 days and in faeces 4 to 18 days post infection. Antibodies could be detected in the faeces for 25 to 58 days. Reinfection of these calves after 50 to 70 days p.i. usually resulted in a secondary response; however, excretion of the virus was not observed after reinfection.

Intestinal defence mechanisms

The resorptive area of the small intestine is exposed not only to infectious agents, but also to toxic and allergic antigens. Hence, the intestinal defence mechanisms are not based on one function only, but are comprised of different protective barriers against such antigens and agents, both specific and nonspecific in nature.

Mucous secretions may function by physically trapping microorganisms, thereby preventing them from reaching the enterocytes. In addition, the mucus itself, a glycoprotein, may contain sites to which bacteria can attach. Lysozyme, which is actively secreted into the mucus, interacts with sialic acid residues in the mucus, thus acting against muramic acid-containing cell walls of bacteria (Clamp, 1981). Lactoferrin is believed to form a complex with iron from secretions, thereby depriving essential iron from the use by bacteria. Glandular secretions from the stomach, duodenum, pancreas and liver supply large amounts of sterile fluid to the ingesta. In co-operation with peristaltic activity, they act by washing out enteropathogenic microorganisms from the proximal part of the small intestine. The inactivating effect of bile salts for enteropathogenic viruses, such as TGE virus, has been shown experimentally (for review see Bachmann and Hess, 1982).

In addition, interferon is produced in the small intestine after viral infection. It is possible that other nonspecific mechanisms influence the course of infections in the gut. The precise effect and the protective value of all these mechanisms on the actual infection with enteropathogenic agents, however, can only be speculated upon.

The main role of the small intestine, in addition to its resorptive activity, consists of its enormous capacity as a lymphoid organ. Lymphocytes are found in Peyer's patches and along the intestinal mucosa. Peyer's patches contain a population of about 80% B-cell and 20% T-cell precursors. The lymphocytes of the epithelial layer are exclusively T-cells, whereas in the Lamina propria, immunoglobulin producing B-cells predominate, the majority of which secrete IgA and only a small number IgM antibodies. The exception are rumi-
nants, where IgG1 producing plasma cells also occur in large numbers (Kemler et al., 1975; Newby and Bourne, 1976).

Antigen stimulation of both B- and T- lymphocyte precursors takes place in the Peyer's patches. Here, specialized epithelial cells, the membrane or M cells are responsible for the uptake of the antigen. As a result of this stimulation, activated B and T cells migrate to local lymph nodes and reach the blood circulation via the thoracic duct. After migration and differentiation, the B and T cells 'home' as mature plasma cells, primarily back to the Lamina propria of the intestine, but also to other mucosal sites including the mammary gland and bronchial tissue of the lung. Migrating T cells 'home' to intraepithelial (IE) sites of the intestinal mucosa. Most of these IE lymphocytes are of the helper phenotype. These cells are probably involved in cell-mediated immunity (CMI), immune tolerance, and in the regulation of humoral immunity.

Consequently, the uptake of an antigen or infection of the intestinal mucosa with an enteropathogenic agent activates a variety of local immune reactions which may either be cell-mediated or consist of secretion of antibodies. CMI reactions include hypersensitivity via helper T cell effects, direct cytotoxicity due to cytotoxic T cells in the Lamina propria, and/or the effects of humoral factors coming from activated T cells, such as lymphokines; all of these mechanisms themselves may lead to various changes in the intestinal wall. Local secretion of immunoglobulins by mature plasma cells, on the other side, usually results in neutralization and elimination of antigens or infectious agents. A schematic representation of the pathway of secretory antibodies is given in figure 1.

After homing to the described locations, plasma cells produce specific antibodies that are stabilized by the incorporation of J (joining) chains. The released immunoglobulins are then bound to a secretory component (SC) which originates from the epithelial cell membrane. Ig-SC complexes are then taken up by the epithelial cells through pinocytosis and secreted into the intestinal lumen, together with an excess of free SC molecules. Secretory immunoglobulins interact with the mucus by means of non-covalent forces, and the Ig-SC enriched mucus covers the epithelial cell surface like a carpet. It thus provides the most effective tool for the prevention of adhesion or invasion of infectious agents. Another source of intestinal antibodies is colostrum and milk from immune or immunized mothers, from which they are transferred to the newborn. These passively transferred antibodies will be dealt with under another topic.

We have investigated the active stimulation of intestinal antibodies relative to their appearance in the faeces and the development of humoral antibodies in the newborn calf. We would like to report on some of these results.

**Materials and Methods**

Five calves delivered by Caesarian section were reared colostrum-deprived and fed reconstituted dry cow milk devoid of rotavirus antibodies. They were kept in individual isolation pens. Two jejunal fistulas were prepared in each calf: one near the middle of the jejunum, the second near the end of the jejunum. Calves were infected orally between 1 and 10 days of age (one day after surgery). Different preparations of the rotavirus field strains Munich V28/78 and Munich V1158/79 were used for infections according to the following schedule: C-4 = V28/78 (K2-42); CsCl gradient purified; ELISA titre 1:3000/0.05 ml; infected on day 10 of life; C-6 = V1158/79; contact infection from another calf; infected on day 1 of life (before surgery); C-8 = V28/78 (K2-42); chloroform-treated faecal material; ELISA titre 1:256/0.05 ml; infected on day 8 of life; C-19 = V28/78 (K2-42); nontreated, but sterile faecal suspension; ELISA titre 1:256/0.05 ml; C-21 = V28/78 (K2-42); infected on day 9 of age.

Each calf was given 1 ml of viral material diluted with 4 ml of phosphate-buffered saline (PBS), pH 7.4. Oral reinfection was performed with homologous virus material, 50 to 72 days following the initial infection. Samples were taken daily from the fistulas, faeces and blood during the first
14 days post infection, and in 3-4 day intervals thereafter. Jejunal fluids as well as faecal material were inactivated at 56 °C for 30 min; prior to inactivation, the faecal material was diluted 1:1 with 0.1 M of sodium acetate buffer (pH 4.5). The determination of rotaviral activity was carried out using an enzyme-linked immunosorbent assay (ELISA) blocking technique described elsewhere (Bachmann, 1979). Immunoelectrophoresis was performed according to the technique described by Grabar and Williams (1953). For determination of Ig isotypes in intestinal fluids H-chain specific antisera to bovine immunoglobulins prepared in rabbits were employed. (Some of these antisera were supplied by Dr T. Newby, Bristol. His help is gratefully acknowledged). (Miles Labs, Frankfurt, FRG).

Results

In all five calves, the occurrence of rotavirus antigens was demonstrated in the jejunal fluids and in the faeces shortly after infection. Furthermore, all calves developed specific antibodies in the small intestine; antibodies could also be demonstrated in the faeces for up to 50 days after infection. Serum antibodies to rotavirus also developed in all calves after infection. The results for each individual calf were as follows. In calf no. 4, rotavirus antibodies first appeared in the jejunal fluid 12 days following infection, but remained at very low levels up to day 25, when the fistula closed. In the faeces, antibodies were demonstrated up to 14 days after infection, after which the level of antibodies remained low (1:10-1:20). The first rotaviral antibody in serum was detected on day 25 p. inf. The animal was reinfected on day 65 after the initial infection, and antibody titers in the faeces and serum significantly increased, starting on day 8 after reinfection. Faecal antibodies, however, remained at high titres for only 16 days, and then decreased to very low titres like those observed after the initial infection. One hundred and seventeen days after initial infection and 52 days after reinfection, faecal antibodies to rotavirus were no longer demonstrable. The high serum titres that developed after reinfection remained unchanged up to day 117 (1:640; 1:1280), when the experiment was terminated.

In contrast to calf no. 4, rotaviral antibodies appeared in calf no. 6 already 5 days after infection, and they increased to very high titres of up to 1:640 on day 10 p. inf. Unfortunately, the fistula closed on day 13 p. inf. Faecal antibodies were first demonstrated on day 18 and reached a peak on day 28 p. inf. 47 days after infection, rotavirus antibodies could no longer be detected in the faeces. In serum specific antibody activity appeared on day 38 p. inf.

Following reinfection on day 50, an antibody shedding profile in the faeces similar to that after the first infection occurred, starting 5 days after reinfection. The presence of rotaviral antibodies in the faeces was no longer detectable 38 days after reinfection (88 days after the initial infection). Serum antibody titres did not increase after reinfection of this animal.

In calf no. 8, specific antibodies were first demonstrated in jejunal fluids on day 4 and in faeces between day 8-10 p. inf. Between days 10 and 20, no rotaviral antibody was detectable in the faeces; instead, virus excretion was observed for three days. On day 21 following initial infection, antibodies were again demonstrable in the faeces, and high titres of activity were present up to day 67 p. inf., when the animal was reinected. In the jejunum, rotaviral antibodies were present between days 4 and 45 at least, at which time the fistula could not be kept open any longer.

Antibodies in the serum were first demonstrated on day 32 p. inf.

After reinfection an eight-fold increase of antibody titres in the faeces occurred after ca. 10 days. The high antibody titre (1:320) decreased constantly, and rotaviral antibodies could no longer be detected in the faeces after day 99 following the initial infection (i.e., 32 after infection). Serum antibody titres rose about eight-fold approximately 10 days after reinfection and titres remained unchanged until the end of the experiment on day 110 (fig. 2).

In calf no. 19 virus excretion was demonstrated in the faeces on day 5 p. inf., and the virus antigen was present in jejunal fluids between days 4 and 7, and again between 15 and 20 days p. inf. Mild diarrhea occurred on days 6 to 15 p. inf. Rotaviral antibodies in jejunal fluids and faeces were first observed 8 days after infection. In serum antibodies first appeared 19 days p. inf. Faecal antibody shedding ceased on day 54 after infection.

![Fig. 2.— Comparison of antibody development in jejunal fluid, feces and serum of calf no. 8, which was infected orally by chloroform treated rotavirus V28/78.](image-url)
After oral reinfection on day 72, high levels of antibody could be demonstrated as early as 5 days p. inf., while serum antibody titres remained low. Faecal antibodies were no longer detectable 112 days after initial infection and 40 days after reinfection. The experiment was terminated on day 117 (fig. 3).

Calf n° 21 excreted the virus for three days after infection, starting 48 h p. inf. The rotaviral antibody was first detectable in the jejunal fluids on day 3 p. inf. and in the faeces on day 4 p. inf. 10 days after infection, serum antibodies appeared, with low titers. Faecal antibodies were present up to 42 days p. inf. Reinfection was carried out on day 50, the first antibodies were found 4 days later. Serum antibodies also increased slightly. Faecal antibodies were detectable up to 95 days after initial infection and 45 days after reinfection. The experiment was terminated on day 118.

Isotype determination of antibodies in jejunal fluids and faeces

In jejunal fluids IgA, IgG1 and IgM could be found. In most calves, IgG1 was the dominating isotype with rather high amounts of IgA also present. In calf n° 4, IgA dominated over IgG1, and in some samples from calf n° 6, IgM could also be regularly demonstrated, but usually only in small amounts.

IgG1 was the dominant isotype in the faeces of all calves. It could be demonstrated throughout the experiment, regardless of whether specific rotavirus antibodies were present. IgA was also present in most of the samples, but only in low amounts. IgM could usually be demonstrated at the beginning of the experiment.

Discussion

Oral infection of colostrum-deprived, fistulated calves with bovine rotavirus resulted in the appearance of local, intestinal antibodies in all animals. However, considerable variation was observed between individual animals with respect to both the onset of antibody production and to the amount produced. Antibodies could, for example, be detected first in the jejunal fluids as early as 3 days p. inf. (calf n° 21) and as late as 12 days p. inf. (calf n° 4). In other calves, they appeared 4 (calf n° 8), 5 (calf n° 6) and 8 days (calf n° 19) p. inf. Although antibodies in the faeces could be demonstrated shortly after they appeared in the jejunum, it took 13 days in calf n° 6 and 5 days in calf n° 8 between first antibody detection in jejunal fluids and in faeces. Similar observations were made with respect to duration of antibody shedding in the faeces and with the onset of serum antibodies. Apart from certain individual variations and variations in the incubation period, which occurred within certain limits, these differences are likely due to physiological and technical reasons. All calves, except calf n° 6 were infected 24 h after surgery. Some of the calves may have recovered more rapidly than others, which is reflected in the uptake of more milk and faster normalization of intestinal functions, e.g. peristalsis. Indeed, calves drank between 500 ml and 1 500 ml per meal, and this may have a significantly different dilution effect of secretory antibodies in the small intestine. In addition, the ELISA technique employed may not have been sensitive enough to detect very small amounts of antibodies in the jejunal fluids and faeces. In future experiments, these problems must be taken into consideration.

Surprising was the late appearance of serum antibodies in four out of five calves. This could be due to the relatively low virulence of the rotavirus strains employed, which only weakly stimulated the development of humoral antibodies. According to present knowledge one would expect, however, a correlation between onset of local and humoral antibody production; this was not the case in these experiments. The discrepancy must be investigated further, especially when employing virus strains with different levels of virulence. Since neutralizing rotaviral antibodies cannot be measured by ELISA methods, it can be speculated that neutralizing antibodies perhaps appear much earlier in the serum.

The longevity of the antibodies present in the faeces varied, but gave preliminary first evidence of specific antibody secretion profiles in the intestine. Antibodies could be demonstrated between 25 and 58 days after onset or between 42 and 67 days after initial infection. In three out of five calves (calves n° 6, 19, 21), rotaviral antibodies decreased to undetectable levels 47, 52 and 42 days after infection, an indication that the duration of rotaviral antibody secretion is limited of be-

Fig. 2. — Comparison of antibody development in jejunal fluid, faeces and serum of calf n° 19, which was infected orally by a 450 nm filtrate of faeces from the second calf passage of rotavirus V28/78.
tween 40 and 50 days p. inf. These conclusions were confirmed by the decrease of rotaviral antibodies in all calves also after reinfection. The time between the onset of detectable antibody development and its decrease to undetectable levels in the faeces ranged from 32 to 52 days (mean: approx. 38 days). These results clearly show that antibody secretion in the small intestine is limited, and reinfection (inducing another period of rotaviral antibody secretion) is possible. It would be interesting to see whether this pattern continues with additional infections, which would imply that animals immune to rotavirus infection must be infected frequently, and that active local immunity is only short.

It is worth noting that calves n° 4 and 8 which still had rotaviral antibodies in the faeces at the time of reinfection, also responded with an increase in local antibody production. Whether this is due to a new infection of enterocytes or a stimulation of the already existing B cell population in the Lamina propria must be investigated in future trials.

Whether the antibody response after reinfection is an indication for a secondary response cannot be answered by these results with certainty.

The onset of antibody production seems to begin at the same time (4 to 10 days p. inf.) as after primary infection, however, antibody titers are higher than after the first infection. This could indicate an immunological memory, but the evidence shown here is not very strong.

The presence of IgG1 and IgA in the intestine and faeces of calves has been shown earlier (Newby and Courne, 1976; Porter et al., 1972). It can either be locally produced or originate from a «spill-over» of serum immunoglobulins. However, the «spill-over» theory certainly does not apply for these experiments with regard to rotavirus antibodies. At the time when rotaviral antibodies were found in the jejunum, specific serum antibodies were not present. On the contrary, when high levels of antibodies were found in the serum, there were fairly long periods when rotaviral antibodies could not be found in the faeces. We believe, therefore, that specific antibody activity, which was detected in the faeces, originates from local intestinal antibody production and not from a «spill-over» of serum antibodies.

The experiments were carried out in colostrum-deprived animals. With respect to the immune response, these animals are a rather artificial system; however, so far no data are available on local intestinal antibody responses to viral antigens in larger domestic animals. Thus, the results presented here can only serve as a basis for further investigations in conventional calves.

**References**


