In Vitro and In Vivo Studies of the Mucosal Immune Barrier After Long-Term Small-Bowel Allotransplantation in Pigs Using Cyclosporine

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Introduction

The intestinal mucosal barrier plays a major role in the body's local and general defense systems. Any alteration of the immune barrier can be responsible for severe diseases. This mucosal barrier consists of a large population of different cells, mainly lymphocytes, plasmocytes, macrophages, polynuclear cells, and mast cells (ARNAUD-BATTANDIER 1984), a fact which distinguishes small intestinal (SI) transplantation from that involving other organs. Surgical improvements and drug developments now enable long-term survival of animals that undergo SI transplantation, including large animals such as pigs (RICOUR et al. 1983) or dogs (COHEN et al. 1983; RAJU et al. 1984) and rodents (THIEDE and DELTZ 1978; SCHRAUT et al. 1983; see review by KIRKMAN 1984). Before the use of immunosuppressive drugs, rejection of small-bowel allografts in experimental animals occurred within 10 days.

Considering the importance of the mucosal barrier in homeostasis, studies were undertaken in animals with total small-bowel allotransplantation under cyclosporine A (CsA) therapy following a two-step procedure: A first set of experiments was designed to evaluate in vitro the morphological characteristics and functions of the lymphoid cells isolated from the transplanted gut; a second set of experiments was performed in vivo after intestinal infection with two different viruses. Both in vitro and in vivo results demonstrated preservation of the mucosal immune barrier.

Materials and Methods

Animals and Transplantation

Eight Large White Pigs (weighing 20–60 kg) were used for transplantation as described by REVILLON et al. (this volume). Briefly, SI segments (6 m long), histoincompatible for one haplotype, were immediately grafted in continuity with the small intestine of the recipient ($n = 4$). CsA (started 48 h before surgery) was given daily intravenously the first 15–20 days following surgery, after which it was administered orally. Animals were used for immunologic studies 10–18 months after transplantation.

We used 34 normal Large White Pigs for the in vivo experiments. They were divided into four groups, with two groups receiving CsA orally (20 mg/kg) and two

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groups undergoing no immunosuppressive therapy, as previously described (BERNARD et al. 1985).

Isolation of the Lymphoid Cells from the Transplanted Gut

Tissue samples were surgically obtained 10–12 months after grafting. Lymphocytes from blood and mesenteric lymph nodes (MLN) were isolated as described by SALMON (1982). Lymphocytes from the gut were isolated by mechanical procedures (SALMON and ARNAUD-BATTANDIER, submitted for publication). Rosette formations with different compounds were used for study of T and B cell surface markers according to SALMON (1982). Histocompatibility tests were performed using anti-swine leukocyte antigen (SLA) reagents as described by RICOUR et al. (1983).

Proliferation studies were performed after lectin stimulation (PHA, Con A, PWM). Interleukin 2 (IL-2) production was tested after a 48-h culture in the presence of PHA and was then tested on a mouse CTL line (ARNAUD-BATTANDIER et al. 1985).

In Vivo Infections with Enteropathogenic Viruses

Immunization with Sheep Red Blood Cells
Fourteen days after the beginning of CsA treatment each normal animal and each transplanted animal received an intraperitoneal injection of sheep red blood cells (SRBC).

Experimental Infection by a Swine Rotavirus
One group with and one group without CsA as well as the transplanted animals, were orally infected with 45 ml of a swine rotavirus suspension ($5 \cdot 10^4$ infectious units/ml) 14 days after beginning CsA therapy.

Experimental Infection by a Swine-Transmissible Gastroenteritis Coronavirus
Forty days after beginning CsA therapy, each of the 34 normal pigs and the transplanted animals received one oral dose of the swine-transmissible gastroenteritis coronavirus (TGE) (10^5 infectious units/ml) in suspension.

Blood and Fecal Sample Analysis
Serum levels of antibodies against SRBC and TGE coronaviruses, were monitored every week from day 0 to day 70 by hemagglutination and seroneutralization tests. Titration of TGE virus inducing interferon activity was carried out in vesicular stomatitis virus (VSV)-infected Madin-Darby bovine kidney (MDBK) cells (LA BONNARDIÈRE and LAUDE 1981).

From day 0 to day 70, stool samples were tested twice a week by ELISA for rotavirus antigen levels and for levels of antirotavirus antibodies with corresponding immunoglobul in classes.

Results and Discussion

Morphological Characteristics of Isolated Lymphoid Cells from Transplanted Gut

Lymphoid populations isolated from the transplanted gut mucosa contained a high proportion of T cells (87% ± 5%) with few B cells (2%), and some Fc-positive cells (12% ± 4%), similar to the proportions observed in normal animals. No difference was demonstrated between isolated cells from the donor or recipient MLNs. Granulated lymphoid cells (ARNAUD-BATTANDIER 1982) were equally present in the grafted and recipient gut (approximately 50%).

Study of the SLA phenotypes of the isolated cells from the different tissues showed that transplanted MLNs contained only cells with the phenotype of the recipient, as did the recipient MLN cells. Within the gut, 10%–30% of the isolated cells possessed the SLA phenotype of the donor; this proportion correlated roughly with the proportion of nonlymphocytic cells that contaminated the mucosal cell suspension.

Proliferative Responses to Polyclonal Mitogens

Long-term CsA treatment did not affect the mitogenic proliferation of cells isolated from the donor and recipient MLNs. Mucosal lymphocytes isolated from the transplanted gut were also able to proliferate and to marked quantities of produce IL-2 after PHA stimulation (four times more than the results observed in stimulated blood cells). Similar results were observed in normal pigs.

These results support the success of the earlier use of CsA in SI allografts in piglets (RICOUR et al. 1983). There grafts were well tolerated and CsA well absorbed, as was also demonstrated in dogs (COHEN et al. 1983). In addition, lymphocyte populations present within the gut mucosa did not seem affected, either by the graft or by CsA treatment, in their morphological characteristics or in their functions (i.e., proliferation and IL-2 production). Therefore, it seems that these cells are able to function in the mucosal immune barrier, as confirmed by a lack of infections even more than 18 months after transplantation.

In Vivo Responses to Viral Infections

Experiments were designed to compare the responses of three animals which had undergone transplantation more than 12 months previously with those of the four groups of eight normal animals, of which two received the same dasage of CsA as the transplanted pigs.

To check their capacity for antibody response an intraperitoneal injection of SRBC was given. All 37 animals had anti-SRBC antibodies 10 days after the injection, and the antibody titer reached a plateau value of 2.5 $\log_2$. Therefore, CsA did not alter the capacity for response to an antigen injection, and humoral immune response

following viral infections was able to take place and be interpreted. Transplanted animals had a similar response.

Rotavirus antigen excretion in the stools was statistically higher in the two groups of animals that had received the virus, independently of CsA treatment. No significant difference was observed in the transplanted group. Antirotavirus IgG and IgM were practically absent in any group. Only antirotavirus IgA could be detected; controls with or without CsA therapy had the same antibody levels (on day 28 respectively, 16 ± 10 μg/ml and 19 ± 9 μg/ml) as the transplanted animals (Fig. 1) (with a maximum value of 30 μg/ml). CsA did not alter the excretion of antigens in the stools, nor was there any change in the kinetics of the antirotavirus coproantibody excretion.

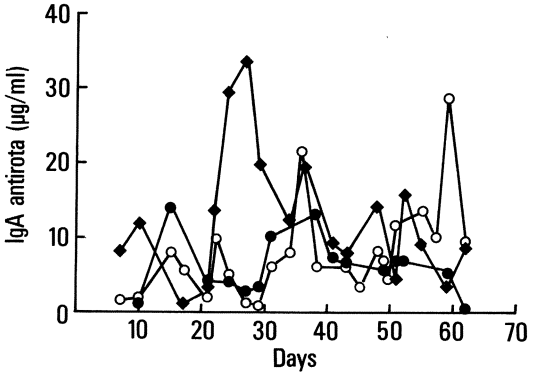

Fig. 1. IgA antirotavirus antibodies in the stools of the three transplanted pigs (○, pig no. 22; ◆, pig no. 60; ●, pig no. 06) following rotavirus oral infection on day 20. Pigs had previously been in contact with the virus, as shown by the presence of antibodies before day 20

After TGE coronavirus infection, only four controls had slight diarrhea. Interferon activity was observed between days 2 and 4, while neutralizing anti-TGE coronavirus antibodies appeared 9 days after oral virus administration and reached a plateau on day 20 (titer 1/128) in all groups. The transplanted animals presented no clinical manifestations, nor were their biological responses different from those of the controls (Fig. 2).

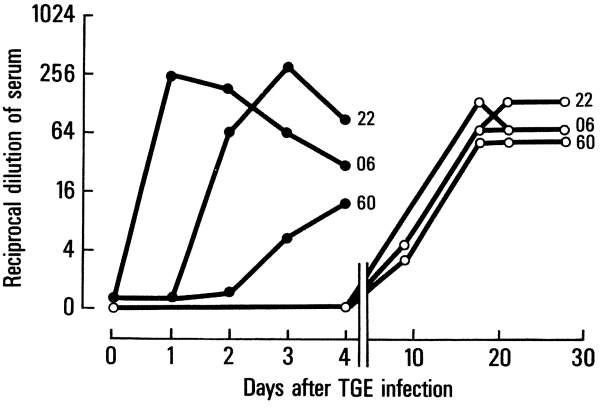

Fig. 2. Serum interferon activity *(left panel)* and anti-TGE coronavirus antibody titer *(right panel)* following TGE coronavirus oral infection (*22, 06* and *60* are the code numbers of the three transplanted animals)

Oral CsA did not alter the proliferation or IL-2 production of cells isolated from the gut mucosa of transplanted small intestines. There was also no difference observed in the humoral immune response against enteropathogenetic virus. Different responses were observed after infection with vaccinia virus or herpes simplex virus, depending in part on the activity of T cells; it has been demonstrated (Cole et al. 1983) that CsA is responsible for a decrease in the animal resistance to these viruses.

To our knowledge, no information was previously available on the role of CsA in viral enteric infection. Our results seem to indicate the absence of CsA influence on the antibody response of pigs to the two swine-specific enteropathogenetic viruses.

Intestinal allotransplantation also did not alter the ability of the mucosal immune barrier to react against viral infections. Therefore, in vitro and in vivo studies demonstrated that the mucosal immune barrier is preserved after intestinal transplantation, bringing in a new argument in favor of human intestinal transplantation.

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