The intestinal barrier and its relationship to pediatric infections *

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Introduction

When you think about pediatric surgery and the development of it as a unit develops as technique and training increases, an then when you sit by and analyze complications in pediatric surgery, it becomes pretty apparent that most of the problems are no longer the wrong or bad operation, anastomosis no longer leaks, the wound heals well.

However, when you look at the causes of death in major pediatric surgical problems, you mainly see two things: the congenital abnormalities that cannot be corrected and secondly, no matter how well developed antibiotics are and how careful technique is, infection. Today, as well as in the past, and almost in every country, surgical infections, particularly in the newborn, and particularly in the premature newborn, have continued, and in some ways and in some cases seem to be even worse. We see this now as a major problem and one of the limiting factors even in transplantation surgery.

Although we are not preventing infection, I think the actual mechanism of septicemia, gram negative septic shock, multiple organ failure, is beginning to become a little clear. It appears that, infection begins by a source, either by bacteria getting into the body by what we call exogenous, by a break in the body through a wound or an intravenous needle or a total parenteral nutrition (TPN) catheter; or by bacteria getting in through the body itself, particularly through the gastrointestinal tract, called endogenous. Once the bacteriae get in, they do not injure or destroy tissue. The body is injured by the violent reaction between the bacteria themselves and the body's immune defenses. One of the messengers that begins this inflammatory reaction including its byproducts, resulting in tissue destruction is endotoxin. Endotoxin is released from the gram negative bacteriae. Macrophages and other immune cells respond immediately, talking to themselves, sending messages back and forth.

They do this by releasing small chemicals called cytokines. Cytokines are messengers that instruct the immune cells to do certain tasks which are prepared to do that. Among these cytokines is Tumor Necrosis Factor (TNF). The reaction between lipopolysaccharide (LPS or endotoxin) and TNF initiates many cycles, and byproducts of these cycles injure the cells. One of them is interleukins, 1 to 12. These are byproducts of the released cytokines by the cells, particularly by the lymphocytes, that have specific reactions on tissue.

Also, bacteria and endotoxin begin to kick of various cycles, among them, the complement system which has elements that injure capillaries and injure cells directly, and Platelet Activating Factor (PAF) which is a cytokine that can kick off the leukotriens and prostoglandins. So that basically, what people find is, in 50 % of the babies that die of gram negative sepsis, there were no bacteria left in the body by the time they die. The bacteria initiate this cascade of reactions that then is almost self sustaining. Then gradually many organs fail and the patient dies.

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Gut as the source

What I would like to talk about is one source which appears to be particularly important in the initiation of sepsis, particularly gram negative sepsis, in the critically ill infant and child, and that is the gastrointestinal tract. The gastrointestinal tract has literally millions of bacteriae within it. Yet, these bacteriae are kept from getting into the body and invading. The gut barrier, or the barrier between the bacteriae and the inside of the body, is very effective in keeping these bacteriae out.

Intestinal mucosa is the barrier that the bacteriae have to get across, much as in the ancient cities, where people built the wall around the city, and for the invaders to get in, they had to either break through the wall or in some way drill through the wall in order to get on the other side to make damage. If you think about this whole complex, that is the gut barrier. Actually, the gut barrier is not just the wall. The bacteriae have to get through gastric acid, gastric motility, the local flora, the immunoglobulins, mucus and the mucosal cell itself. If the bacteriae get through all of these, then they can cause sepsis. These hurtles altogether are called the gut barrier.

Actually how the bacteriae get through is only beginning to be understood. Some people think that, what happens is the bacteriae come up to a mucosal cell, blow a hole in the cell and get through. Perhaps that is one mechanism. Some people believe that, they push the two mucosal cells aside and get in through the junction between the cells. It is known that, for a bacteria to get through mucosal cell, first thing it has to do is to stick to the mucosal cell, and it actually has receptors that much as a key goes into the lock, joins against the mucosal cell and then passes through. So, this first process, that bacteria has to get against the cell to get in is called adhesion.

Components of the gut barrier

Let's take a baby and put him in an intensive care unit (ICU) and think about the gut barrier. The first thing happens is, one puts in a nasogastric (NG) tube and then the bacteria living in the ICU, which are usually very pathogenic bacteriae, walk down the NG tube, and end up in the stomach. The stomach secretes gastric acid and kills many of the bacteriae. However, we worry about stress ulceration and in order to prevent it, in many ICUs, the first thing we do is to give the patient H2-receptor blockers to decrease gastric acid. But by the same token we now allow these bacteriae without gastric acid to grow in the stomach and pass on down the gastrointestinal tract. So, the first part of the gut barrier is really gastric acid.

The next and very important mechanism for the gut barrier is intestinal motility or peristalsis. As the bowel squeezes it moves the bacteriae down the gastrointestinal tract and finally out of it. But the sick patients in the ICU, especially when we operate on them, develop ileus. With ileus, there is statis and overgrowth of bacteriae. That is the second part of the gut barrier.

A third aspect and an extremely important one is the secretion of mucus from the goblet cells in the intestine. In order for the bacteriae to pass through the mucosal cells, they have to get right against it to adhere to it. But the goblet cells secrete mucus and trap the bacteriae in this layer of mucus and keep them from adhering to the mucosal cell. The sick patient in the ICU, particularly who might have got pilocarpine or atropine for operation, has very little mucus. Babies, particularly prematures, have a defect in mucus secretion. So, we have another vulnerable point with the patient in the ICU.

Probably one of the most effective ways of stopping bacteriae from adhering to the mucosa is IgA. IgA is secreted by the gastrointestinal tract. Bacteriae adhere to the mucosal cells by receptor sites. IgA binds these receptor sites on the bacteriae. So, the bacteriae cannot stick to the mucosal cells and these bound bacteriae pass through the gastrointestinal tract. The baby is not born with IgA and the only way to get it is breast milk and colostrum. So, it becomes extremely vulnerable to adhesion by bacteriae and passage of bacteriae through the mucosa.

In the gastrointestinal tract, there are bacteriae living in the crypts of the mucosal cells. They are a kind of friendly bacteriae forming our resident flora. Replacement of the flora by pathogenic bacteriae indicates colonization. The resident bacteriae have cer-

tain mechanisms to resist colonizing pathogenic bacteriae, such as releasing antibioticlike substances to kill them. Unfortunately, when we put a baby in the ICU, we given him antibiotics whether he needs it or not that kills all of these weak but friendly bacteriae, and allows the resistant pathogenic bacteriae to replace the ones that live there. So that actually, the misuse of antibiotics sets us up a colonization by pathogenic bacteriae by killing our benign resident flora.

Methods of studying gut barrier

A. Clinical

1. A very simple and widely used method is to get cultures simultaneously from the gut and blood. If a bacteria can be found in specific quantities in the gut and if at the same time we can show the same bacteria in the blood, one would suggest that perhaps bacteria goes from the gut to the blood.
2. We can study and document specifically the gastrointestinal flora.
3. We can use the technique called selective bacterial decontamination.

B. Translocation

It is the experimental technique used in the laboratory which can be defined as the passage of living bacteriae from the gastrointestinal tract across the intestinal mucosa. You culture bacteriae in the stool and then you culture bacteriae in the draining lymph nodes. If you find bacteriae growing in the mesenteric lymph nodes, you then assume that there was bacterial translocation, which means bacteriae have passed through the mucosa, through the lympatics and into the lymph nodes. So that experimentally, if you get a positive lymph node you can say that the patient had translocation. If at the same time the patient also had bacteriae in the liver, or spleen, you can say that he also had sepsis or septicemia. This is an experimental technique and usually done on rats. You can look at their lymph nodes which usually should be negative. They may have as much as 10 % translocation, or you can, for example manipulate their intestine and, if you then you sacrifice them and study the lymph node, you find an about 60-80 % of times of lymph node positivity.

C. Ussing chamber

Ussing chamber is developed by a biologist to study passage of various substance across living tissue. We simply put the living membrane, the intestinal mucosa of the rat obtained by stripping off, between the two halves of the chamber. After it is closed the only thing separating these two chambers is the living mucosa. We can bubble nutrient solutions and oxygen to keep membrane alive and healthy. We put electrodes across so that we can measure the potential difference in the membrane which tells us whether the membrane is alive or dead. We can keep the mucosa alive for a few hours. We can put bacteriae on one side and serially measure the passage across to the other side to be able ex vivo to study the passage of bacteriae in the mucosa. We can take samples of it to study under the electron microscope.

III. Research pending

A. Clinical

We got interested in the clinical significance of the gut as a source of infection when we started with Dr. Starzl to the first few multivisceral organ transplantation with cyclosporine. The first patient who had multivisceral transplantation was studied well. Almost immediately after transplantation she developed severe Serratia septicemia. It was obvious that although the bowel was not rejected, it became permeable to bacteriae. During 193 days she lived, she had 46 positive blood cultures, and had 14 proven septic episodes where she had all the signs of clinical infection. We simultaneously studied the bacteriae and her transplanted bowel when we got blood cultures. In 13 out of the 14 septic episodes (93 %) the same bacteria first would be isolated in the bowel and within 24-72 hours, that same bacteria would than appear in blood. So that, the bacteriae were rapidly passing through the intact mucosa which we recurrently biopsied, and causing septicemia.

In Pittsburgh we have a very large group of patients with short bowel syndrome. These patients are on home TPN. One of our problems with these patients is they recurrently get septicemia. We postulated that, perhaps since they were in short bowel syndro-

me, the bowel becomes large and distended, so there is bacterial stasis, there is overgrowth of bacteriae, translocation of them into the lymph nodes and then into the blood stream, where they then deposit on the catheter. So, one of the reasons that we have a high incidence of TPN catheter sepsis in patients with short bowel syndrome is the gut as a source of bacteriae. We looked at all the catheter sepsis with short bowel syndrome and compared them with similar patients without short bowel syndrome.

Our incidence of catheter sepsis was 7.8/1000 hrs of TPN for short gut patients, while it was only 1.3/1000 hrs of TPN for controls. We also found that the control patients mainly had infection from skin bacteriae, particularly the staphylococcus. The short gut patients mostly had enteric bacteriae, such as Pseudomonas, or E.coli. At 68 % of them, the same bacteria that apeared in septicemia also was in the stool. They usually did not have anaerobic bacteria in their intestinal tract. At some of the patients we had to operate on, we biopsied the mesenteric lymph node. At 75 % of them, same bacteria grew in the lymph node, suggesting translocation.

We got involved with selective bacterial decontamination (SDD) which was developed in Gröningen, with the first patient with small bowel transplantation. The Gröningen group said that it is impossible to completely sterilize the gastrointestinal tract, because bacteria would keep growing and they would be more resistant. So, the thing to do is not decontaminate the bowel, but selectively kill bacteriae and leave other ones in. In other words as we say in the States, you kill the "bad guys" and leave the "goods guys" in. The "bad guys" appear to be gram negative bacteriae, particularly the pathogenic bacteriae such as Serratia, Klebsiella and Pseudomonas. And the "good guys" appear to be anaerobic bacteriae because, they appear to be the bacteriae that try to prevent the gram negative bacteriae from colonizing the gastrointestinal tract. So, SDD is using nonabsorbable bacteriae to kill off the potentially pathogenic bacteriae and allow the resisting bacteriae to live.

The Gröningen Group to SDD by giving a mixture of polymyxin, tobramycin, and amphotericin-B down the gastrointestinal tract through the NG tube, 4 times a day. They also put it on the oral mucosa in an orabase, so that, it trickles down through the esophagus into the stomach. They use cefotaxime as an antibiotic just for the few days until the bacteriae having a change to get hold. This is the regime they use, and many people have used this in ICU and with transplantation.

We have done two clinical studies both on liver transplantation with SDD. The first one was with cyclosporine and second with FK 506. One group would get SDD and the second would not. We had 188 SDD patients. The ones that got SDD had no sepsis from Gram negative bacteriae, while there were 6 proven Gram negative infections in the control group. Gram positives and fungi were reduced as well.

It takes about three days for the bacterial flora to change. If you start SDD postoperatively, you get a three days with bacteriae haven't changed. So, in our last study with FK 506, we started the antibiotics preoperatively and we also used a solution to mechanically cleanse the bowel called Golytely by whole bowel irrigation.

B. Translocation

We made a study in which we tried to see if SDD is effective in preventing translocation in the rat. If you take rats and operate on them and handle their bowels in the laparotomy, 90 % of them have a positive mesenteric lymph node. If you mechanically cleanse the bowel by using Golytely, you can reduce translocation to 25 %. If you give SDD before you manipulate the bowel, you can reduce it to 10 %. If you can bind mechanical preparation plus SDD you can reduce translocation to 0.

In another study, we wanted to see if it really is true that transplanted bowel translocates. We did small bowel transplantation in rats. We used inbred strains of rats, so that they did not reject.

In those, 4 out of 10 animals translocated through transplanted bowel, which suggests that if you have an autotransplant, even if your own bowel, just the trauma from the operation will cause some translocation. If you transplant from a nonrelated animal,

so that they will reject, in 7 days while the bowel still looks well, almost every one of them will translocate. So, when the bowel is rejecting, there is no question that translocation occurs. However, if you use Cyclosporine or FK 506, even though the bowel looks absolutely normal, and functions well, almost every one of them translocates. So, right after the bowel is transplanted, at least for a week or two, the bowel, in spite of looking normal by the microscope, translocates vigorously.

It is not just how many bacteriae there are, but also what kind of bacteriae they are. In another experiment, we used E.coli-K1 which causes immediate septicemia and meningitis in newborns. We also used E.coli-K 100 which looks almost identical to K1, but a perfectly benign bacteria. If you take newborn rabbits and you put K1 in their intestinal tract, you find about 50 % of them will grow bacteriae in their mesenteric lymph nodes, which suggest that K1 gets into the body of the baby through the gastrointestinal tract. If you give K100 in large amounts to the baby rabbit, it also goes almost the same amount of time to the mesenteric lymph nodes. But K1 also goes to the blood, liver and spleen, causes sepsis and death. K100 gets to the lymph node but there, it is stopped by the lymph node and you never see it elsewhere. So, it is not just whether the bacteriae get through the gut, but what kind of bacteriae they are.

C. Ussing

Mucus is an extremely important part of the gut barrier. If you give pilocarpine to the animal, which stops mucus secretion, and then, place the membrane in the Ussing chamber and put bacteriae to the mucosal side, 75 % of them go across. However, if the animal is able to secrete mucus, as in the control group, only 27 % passage takes place. So, this experiment shows that, mucus is a very essential part of the gut barrier.

It has always been told that IgA binds the bacteriae and does not allow them to stick to the mucosa and pass. We showed experimentally for the first time that, if you put bacteriae in the Ussing chamber and then add IgA, there is absolutely no passage across the mucosa. If you don't give IgA, you get 60 % passage. So, this looks like a very striking method of preventing passage.

We looked at newborn versus older piglets to see whether the baby's mucosa is permeable or passes more frequently than does the older's mucosa. We found that, there is a much higher incidence of bacterial passage in the newborn than in the animal that is already eating and weaned from breast milk.

If you put E.coli in the ussing chamber, about 42 % passage will take place. However, if you put Salmonella which destroys the cell and gets through, you get 92 % passage, which evidently shows different effects of unlike bacteriae.

Passage of E.coli requires an active energy consuming process, without injury the mucosal cell. After being phagocytosed by the mucosal cell into small intracellular vacuoles, most of them are killed intracellularly. However, some living bacteriae are passed to the other side of the cell, where it then is phagocytosed by a macrophage. This marks the beginning of an antigenic sampling process, taking place in the gut of the pregnant mother. Eventually, IgA is produced and secreted in breast milk, which already knows E.coli and binds it in the neonate's gut. So, this is the reason why some of the bacteriae are allowed to pass to the lymph nodes, while many are killed, in a normal organisms. If you deprive the mucosa off oxygen or make it cool, which reduces metabolic activity, then passage is reduced. Likewise, if you put glutamine, which has been shown to be the best fuel for mucosal cells of the gastrointestinal tract, to the chamber, you get an increased passage of E.coli.

IV. General comments

Breast milk

Breast milk has IgA in large amounts, which has been specifically prepared by the mother against the bacteriae that she has been exposed to. It binds bacteriae, so they cannot adhere to the mucosal cell. Breast milk also has epithelial growth factor which causes growth and maturity and gives resistance to the mucosa. So, the mucosa of the babies becomes more mature when they get breast milk. E.coli and

many other bacteriae in the gut cannot live unless they have iron. Breast milk contains lactoferrin which specifically binds iron. So that, without available iron bacteriae die. Breast milk has bacteriae such as lactobacillus and bifidum which can colonize the baby's gastrointestinal tract and resist pathogenic bacteriae. There are also oligosaccharides in breast milk which can bind directly to the receptor sites on the bacteriae, preventing adhesion of bacteriae to the mucosal cells. So, it is obvious that breast milk has many factors that allow the gut barrier to function effectively.

Pathogenesis of necrotizing enterocolitis

Contrary to general expectation, we are beginning to see more and more NEC patients. With better intensive care and the use of surfactant, we are having more vulnerable babies, who stay alive and then become vulnerable for NEC. Let's think about a small premature baby who has respiratory distress and goes to the newborn intensive care unit. The baby has never had breast milk, he may be had formula. This is an immature baby and has immature mucosal cells.

Usually he has a high gastric pH or we place a NG tube in and pull out the gastric acid or give him H2-blockers. Premature babies have less secretion and less effective mucus to stick. They do not secrete IgA and if they haven't had breast milk, they have no IgA. Now, you have an extremely vulnerable patient with very few of the aspects of the gut barrier. When you put him in the ICU, you put a NG tube down, you touch him, you breathe on him, you put him in the incubator and blow bacteriae at him. So, potentially pathogenic bacteriae begin to go down the gastrointestinal tract.

The next thing that happens is, you feed the baby a little bit of formula, which is just the right food for bacteriae to grow. So, these bacteriae overgrow and since most premature babies, particularly if they had surgery, have ileus, you now have stasis. You have overgrowth of bacteriae with substrate in a vulnerable patient. Then you get colonization in the baby's gut and then the bacteriae go across the mucosa. Now, there is inflammatory reaction in which the bacteriae, as they go across, meet the macrophage, and, TNF, PAF, various aspects of the kinin system, and the complement system are released which cause damage to the intestinal wall including the mucosa. This allows more bacteriae to go across and then you have, what we would clinically call, NEC. Perhaps, NEC is not a specific disease, but almost what we seen in the adult which is called multiple organ failure.

You have a vulnerable host, who then becomes colonized by pathogenic bacteriae and when they get in a high enough concentration, they can move across the intestinal barrier, cause an inflammatory reaction and the products of this reaction begin to cause a smoldering fire which causes more damage, and then this progressively goes on to bowel necrosis. As a conclusion, the things that we need to look at are, less contamination of these babies and more defence and support of their gut barrier. Because, it has already been shown in a very nice study that, by giving IgA by mouth, the incidence of NEC has been significantly reduced, which again suggests that, the gut barrier is an extremely important factor in this subject.