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Discussions

DR. EDWIN DEITCH (Shreveport, Louisiana): As you have just heard, the authors have presented data indicating that brush-border glutaminase activity and the transport of glutamine is decreased, and this decrease appears to be due primarily to quantitative decreases in the amount of glutaminase enzyme or glutamine transporters and not due to functional alterations of these systems. That is, what is there works. The problem is that what is there is reduced in number.

This basic work has very important clinical implications because we have information indicating that glutamine has several unique and important functions. We know that glutamine, rather than glucose, is the primary nutrient to the small intestine and that maintenance of intestinal structure and function may depend significantly on glutamine availability.

In addition we know that glutamine is an optimal and essential nutrient that's required for the immune system to function. Thus one clinical implication of these results is that the net effect of these impairments in intestinal glutamine metabolism and transport may result in an inadequate availability of glutamine to support optimally intestinal barrier functions during stress states.

This assumption fits in very well with the previously published data from my laboratory looking at endotoxin and barrier function of the intestine. We have shown, and others have as well, that endotoxin in stress states can lead to gut barrier failure and potential lethal gut origin septic states.

Would the authors comment on the potential therapeutic role of glutamine administration? I ask this question because the second implication of this work is that because the glutaminase and glutamine transporters that are present function normally, it may be possible to meet the cells' glutamine needs by increasing substrate availability.

In other words, it may be possible to compensate for these defects in glutamine transport by increasing the amount of exogenously supplied glutamine.

Because the physiology of glutamine metabolism and transport *in vivo* is incredibly complex and is influenced by more variables than can be addressed by *in vitro* studies, I would like to ask the following questions.

In vivo glutamine is transported to the cells from both the blood *via* the basal lateral membrane as well as to the intestine across the brush border. Thus would you put your data on luminal transport into perspective with the information you have generated on basal lateral transport? This question is potentially important because, especially in a nonfed state, the majority of glutamine transported to the epithelial cells may come from the blood.

There are two glutaminases. A phosphate-independent glutaminase, which is in the brush border, and a membrane-dependent glutaminase, which is in the mitochondria.

Because mitochondria glutaminase activity may be more important physiologically than brush-border glutaminase *in vivo* under certain circumstances, do you have any information on whether mitochondrial glutaminase activity is altered?

This question may be important because, methodologically, when the vesicles are formed from homogenized mucosa, it is possible that it could contain or have been contaminated with mitochondria or mitochondrial fragments.

Thus do you have any data documenting whether brush-border glutaminase was contaminated with mitochondrial glutaminase?

Last because glucose, alanine, and leucine transport also were decreased, it appears that the observations of glutamine transport are not specific. Instead they may reflect a global and nonspecific change in membrane transport. Would you speculate on the mechanisms underlying these changes?

DR. J. W. ALEXANDER (Cincinnati, Ohio): I think it's fairly clear from Dr. Copeland's very lucid presentation what the implications of this paper are. That is why it's so difficult to maintain nutritional status by either the enteral or parenteral route in individuals who are septic.

I'll make my discussion relatively short by focusing on four areas of questions, one of which is methodologic. And that is partly because of my ignorance. It would be interesting for all of us to know whether there could be some methodologic variance of this study because of differences in potential fragility of the vesicles as they are prepared. It could be, as an example, that they are more fragile in the septic state. They could create a methodologic error decreasing the apparent transport.

They have shown clearly that glutamine and glucose and aminoacid uptake is decreased, and I would like to know if there is any evidence of decrease of other alternative fuels for the gut, such as short chain fatty acids, ketoacids, or even lipids.

Next is there anything that might reverse this process? This has tremendous therapeutic implications for the septic patient who is trying to be fed. As we know in normal individuals, there are a variety of things

that can increase uptake of the intestines of a variety of substances, particularly nutrients. These include dietary substrates themselves and a variety of hormonal substances such as bombesin and perhaps growth hormone and certainly some of the prostaglanden analogues, particularly of the E series.

Finally, as Dr. Deitch has indicated in his discussion, there is a great amount of data that have accumulated related to transport of endotoxin across the gut, and some of this will be discussed in my paper tomorrow.

It is clear that transport of endotoxin across the intestinal barrier is increased following a variety of injurious stimuli. In the paper Drs. Copeland and Souba have indicated that in fact glutamine uptake increased at 4 hours. Thus there is a definite difference related to the time of the injection of endotoxin, and as to whether uptake is increased or decreased.

Is there an explanation for this change in the transport of these substances? And in particular, might there be a decrease in endotoxin later? Of even more interest, does endotoxin regulate the changes in these transport mechanisms?

DR. JOSEF FISCHER (Cincinnati, Ohio): This is a nicely done and presented continuation of work that shows the critical nature of glutamine to enterocytes.

May I have my first slide, please?

This is some work that really doesn't have much to do with sepsis but shows isolated enterocytes in a different situation, which shows that you can increase the output of protein synthesis in gut mucosa and isolated enterocytes in a control situation and also sometimes substitute for glutamine with various other aminoacids.

We agree with the premise that glutamine is—and much of the work that Souba and Copeland have done shows this—an essential fuel.

They have raised a number of other issues. One of the issues is whether glutaminase is a good marker for overall metabolizing capacity by the intestine. Glutaminase is a funny enzyme, to which Dr. Deitch has already alluded. It exists in liver, brain, and kidney, and it's a very small enzyme. It only weighs about 65 kd. And so there are difficulties in isolation.

Many laboratories use antibody-binding techniques to measure the activity of glutaminase, which is a sure sign that the standard techniques that most of us use to measure antibody activity really don't work very well.

Dr. Alexander has already raised a question of methodology. Whenever I see changes in km or the actual structure or the affinity of an enzyme, I have to wonder whether there is a methodologic problem, although the data in this manuscript seemed pretty good that they are actually seeing an alteration in km.

Without putting any words in the authors' mouths, they have a very attractive hypothesis that decreasing glutamine uptake and decreasing glutaminase results in greater permeability supporting the noxious effects of sepsis by translocation of either bacteria or other products into the gut.

Unfortunately there are scenarios other than endotoxin and sepsis in which this has been tested, and the data are not consistent. Newsholm, for example, has found that in burns—in which I think everybody agrees that there is increased permeability of material across the gut—despite the fact that everybody agrees that in the burn model translocation occurs, there is increased glutamine use and an increased measured glutaminase activity. So there is disparity, at least in the model they used, although it's a little longer term.

The second issue, and this is referred to in the manuscript, is studies by one of our own residents, Dan van Allmen, who found that in sepsis—and this is a cecal ligation and puncture model—protein synthesis by isolated enterocytes at 16 hours actually increases. Now some of these proteins are gut hormones, which we think are exported to the liver, that stimulate the liver for sepsis. So there is that nice relationship. And as the authors have already noted, this is biphasic. And early studies show that these are stimulated.

I would like to ask three questions. First you have studied the vesicle transport in the brush border but not in the basal lateral membrane. What is the relative importance of the basal lateral membrane? I think Ed Deitch already referred to this. And is it possible that there is a disparity between these two types of transport?

The second is a technical question. I assume that their control patients, the seven control patients, were fasted overnight and they took the special pains to make certain that the septic patients were not fasted for any longer than 36 hours. And 36 hours seems like a relatively short time, but in fact it's a relatively long time to the intestine, in which the turnover is 36 hours.

Do they have any data in control patients who have been fasted for 8, 12, 24, 36, and 48 hours? Did the difference in time between the control patients and the septic patients have any influence on the results.

This is fascinating work with important clinical implications.

DR. W. W. SOUBA (Closing discussion): Dr. Deitch asked what happens to transport across the basolateral membrane. We do not know the answer to that question because the basolateral prep is more difficult to work with and the percentage contamination from other organelles is higher. Although we previously reported that uptake of glutamine from the bloodstream is decreased in septic patients, this could be secondary to a decrease in intracellular metabolism rather than a direct reduction in basolateral transporters. Most likely, however, transport across the blood facing membrane is also diminished.

It is important to differentiate between transport and metabolism. One phenomenon involves translocation of glutamine into the cell from the gut lumen from the bloodstream. The second, metabolism, involves the hydrolysis of the glutamine molecule by the glutaminase enzyme, which is mitochondrial bound.

The regulation of these changes is poorly understood. The regulation may be biphasic in nature in that early on transport may be increased (4 hours) with a later decrease in transport activity. Perhaps the cell is reprioritizing protein synthesis during severe infection.

Dr. Alexander asked about methodology. The vesicles from both septic and normal patients appeared to be functional with good vesicle integrity based on similar 2-hour equilibrium points, similar enrichments, and identical diffusion capacities. In terms of recovery from these insults, that information is unknown.

With regard to Dr. Fischer's questions, we are using a human enterocyticlike cell line that allows us to study direct effects of regulators independent of factors such as bloodflow and cell–cell interactions. We are aware of Dr. Van Allman's work from Cincinnati, which shows that protein synthesis in the gut mucosa of septic animals is increased at the 16-hour time point. This fits with the idea that the cell may be redirecting synthetic properties.

Whether decreased glutamine availability results in mucosal injury is unknown. Certainly in other models of bowel injury, glutamine can accelerate healing.