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Discussion

DR. DAVID L. DUNN (Minneapolis, Minnesota): This study represents the culmination of two decades of research focusing on a specific question: Why do surgical patients develop an infection, how can we best identify this, and how can we prevent it?

Over the years, Drs. Christou, Meakins, and MacLean have provided surgical science with a wealth of data in this regard, the interpretation of which may have significant clinical ramifications.

From the current study, we now know that the phenomenon of anergy represents a defect in T-lymphocyte trafficking and, therefore, T-lymphocyte activity in the tissue compartment. This is clearly something that we suspected, but it has now been identified with the current *in vitro* and *in vivo* data.

The authors chose the skin as an easy window to view this phenomenon and infer similar T-cell deficiency in tissues throughout the body. Perhaps not surprisingly, cytokine message in the form of interleukin-3 and interferon-gamma mRNA was not present in the skin of anergic patients because recruited T cells, as Dr. Christou pointed out, were not there.

The importance of this finding, however, I think was somewhat downplayed in both the presentation and the manuscript, which really contains a wealth of data and which I recommend to you. And the importance of the finding is that we really now have a mechanism to explain the phenomenon of anergy. It is very clear-cut what is occurring in these patients.

The clinical findings of this study, however, were mixed. The good news is that so-called walk-in anergic patients do well, probably based on incremental improvements in medical and surgical care that have occurred over this two-decade period. The bad news is that anergy in the surgical intensive care unit patient continues to be associated with approximately a 30% mortality rate. And I would point out to you that this mortality rate demonstrated by complete anergy is very similar if not identical to that of current studies in which sepsis syndrome was studied. I have several questions for the authors.

First, in the intensive care unit, does their study really tell us whether anergy is associated with or a consequence of infection? As Dr. Christou pointed out, this is probably the key question. But my question for him revolves around whether or not low-grade infection was present in some patients.

He clearly demonstrated that this was not the case with regard to bacteremia and intra-abdominal infection. But I think we need to ask the question as to whether or less severe infections concurrently were present. This is an extremely hard question to answer without performance of a large prospective study in which infection surveillance at numerous sites takes place. However, I would appreciate hearing the authors' comments along these lines.

Also, this study brings into clear focus the importance of T cells and the composite immune response to infection. I firmly believe that T cells and macrophages exert numerous effects by directional signaling, probably through cytokine release, an activity that is extremely important in phagocytic recruitment and enhancement of activity of phagocytic cells at the site of infection.

Dr. Christou, please hypothesize how the various components of the immune system function in normal and anergic patients. Is the T-cell portion of the response redundant in most patients, except for those that are anergic? Or is there a spec-

trum of the anergic deficit? Also, have you attempted to perform phenotypic analysis of the T cells? Is there a specific subset missing?

Because this study encompasses such a long time frame, did any of the patients receive levamisole hydrochloride, perhaps at the beginning of your study? That study provided intriguing data and was perhaps the first indication that intervention immunologically was possible. On the other hand, did any patients receive interferon-gamma as part of a more recent study?

Finally, should we continue to view anergy, now to be examined only in intensive care unit patients, I assume, as a harbinger of doom, or can we in fact effectively intervene? If the latter, what interventions would you propose?

DR. JOHN A. MANNICK (Boston, Massachusetts): I suspect that one of the reasons why the authors of this landmark paper sent me a manuscript—in fact, to be perfectly honest, two manuscripts—was that when Lloyd MacLean presented his original paper 20 years ago I was one of the discussants—which shows that some of us have been around a long time in this field.

The paper 20 years ago opened up a new field in surgical investigation, in my opinion. I was very interested in it because our own laboratory at that time had just made some initial observations that after serious injury, lymphocyte activity was suppressed, as we have mentioned before to this group, and here was an *in vivo* confirmation of what we had seen *in vitro,* namely a T cell-dependent phenomenon that was absent in a number of patients and was correlated with death.

This was a stimulus for us to go back and keep on working on the problem and I think was a stimulus for a number of other surgical investigators to pursue this perplexing question of why an injured patient should suddenly have a defect in resistance to infection where all logic would suppose that nature should have increased the defenses against infection under such circumstances.

I am not sure we know the answer to that question yet, but I think that this group, Dr. Christou and his colleagues, have pursued this perplexing conundrum *in vivo* with very innovative techniques—and not only *in vivo,* but *in vivo* in man. And no other group has really got this kind of information.

I have always been intrigued as to the next trick they will use to look at sick patients without harming them and yet come away with an insight into what is going on. And this latest use of the polymerase chain reaction technique to look at cytokine message in a skin test site in a sick patient is really absolutely phenomenal in my view and has answered a critical question, as Dr. Dunn has pointed out. I have only a couple of questions for Dr. Christou.

The first is, I know the T cells from the whole population of anergic patients that you have studied—and I suppose it would be hard to complain that the group is not large enough with more than 4000 patients in it—apparently proliferate okay to things like cellular antigens. What happens in these patients if you stimulate them simply with T-cell mitogens? Is proliferation depressed? Is proliferation depressed in any subset of this vast group of anergic patients?

And finally, because it is clear that cytokine production in the skin at the site of the skin test is depressed, because there are no T cells there, have you gone to *in vitro* studies to learn what the T cells in these patients are making if you stimulate them *in vitro?*

My final question is, because mortality in these elective anergic patients has now gone down to near control levels, how do we explain this? Is it simply that surgical technique has become so refined at the Royal Victoria that patients are not getting infected anymore and that they are in fact something like patients with acquired immune deficiency syndrome, that is, if all goes smoothly they survive surgery, but if you get an infection you are in deep trouble?

DR. BASIL A. PRUITT, JR. (Fort Sam Houston, Texas): I rise to compliment Drs. Christou and Meakins on bringing this important information to our attention and confirming that there has been a general improvement in care of critically ill surgical patients.

I think it is important to point out that the equation defining infection involves both the host and the microorganism. In our burn patients who develop infections, we have noticed that there has been a change in predominant causative organisms from gram-negative to gram-positive.

That change is important, because gram-negative organisms exert a significant comorbid effect on the host and gram-positives do not. So I ask whether you, too, have observed a change in the predominant organism causing infections in your surgical patients.

DR. CHRISTOPHER C. BAKER (Chapel Hill, North Carolina): I also wish to compliment the authors. Because Dr. Mannick went down memory lane, I will say that 20 years ago, when this work was presented, I was a junior resident and Dr. Meakins discussed my paper on T suppressor cells, and his major question was: What about T cell-independent infections? I think it is interesting that the superb work that they have done here has shown the fact that our previous bias was probably correct.

I really need to underline what Dr. Dunn said. I think this work is one of the first demonstrations in humans of the critical difference between looking at serum cytokines as so many people have done and looking at what is happening in the local tissue site in terms of cell trafficking as well as membrane-bound cytokines. I would be interested if you had looked at membrane cytokines in these patients.

One of the other questions I wanted to ask Dr. Christou is, what is the effect of edema on the skin testing, or, more importantly, your new blister technique, in the severely traumatized patients early after resuscitation, because so many of these patients have massive peripheral edema?

Dr. Christou did not talk about the nutritional data mentioned in the abstract, but I would ask if he could say something in the discussion. We participated in a multicenter trial, which Fred Moore presented about a year ago and published in the *Journal of Trauma,* looking at moderately severely injured trauma patients, comparing standard enteral nutrition and an enhanced diet with arginine and glutamine. Although we found many of the same findings in the *in vitro* tests, the striking finding was that there were five intra-abdominal abscesses out of 50 patients in the control group *versus* none in the enhanced diet group. So I wonder if he could make a few comments on that.

And finally, I wonder, Dr. Christou, if you could tell us a little bit about your thoughts or any data that you have on the role of the macrophage. I think you clearly pointed out the critical nature of defects in antigen recognition, and obviously the macrophage role here is fairly critical. Do you have any data in your skin windows on macrophage trafficking in these patients?

DR. NICOLAS V. CHRISTOU (Closing Discussion): I would like to thank the discussants for their kind remarks.

Dr. Dunn asked—if I interpret his low-grade infection question correctly—was infection present in the elective patient group and does infection predispose to anergy and how does this relate to the intensive care group?

It is possible that low-grade infection was present in some anergic patients, because we know that a major infection will produce anergy eight times out of ten, especially in the intensive care patient. The elective patients were admitted for gastrointestinal (GI) resections. Something was wrong with their GI tract which is full of bacteria. In a colon carcinoma, for example, there could be a background level of bacterial toxins absorbed in the blood, making these patients anergic. The role of anergy in outcome speaks for itself. It predisposes patients to a mortality from a subsequent infection.

The reduction in mortality over the past few years, which was addressed also by Dr. Mannick, we feel is probably because of good surgical practice. I do not think that we are unique. A similar study was done by one of our Fellows in Germany, with an equally large data base of about 280 patients, and their mortality for major gastrointestinal resection was very close to ours. I think this is a generalized phenomenon.

What exactly is the role of the T cell, is what Dr. Dunn asked, in this situation? What I think is happening is the following. Lymphocyte recirculation through the high endothelial venule occurs normally in these anergic patients. Lymphocyte recirculation through the skin appears to be abnormal, for whatever reason. We do not know the mechanism for this. Memory T cells that normally patrol the skin are not there to detect antigen, and therefore initiate a lymphokine response supposing that antigen was a single bacterium. One memory T cell will respond to one bacterium. The nonspecific host defense will not respond to just one bacterium. There has to be quite an insult for that component to kick in, if you wish. We speculate that in the anergic patients the "alert" of the antigen specific T-cell system required for nonspecific immunity to be initiated and attack the invading organism may not be occurring. If the same holds for the gut, it would render anergic patients susceptible to invading pathogens.

Patients receiving drugs such as levamisole were excluded from the database. The same goes for antitumor necrosis factor-alpha in the intensive care unit. Therefore, there is no impact of drugs in what we presented here.

Dr. Mannick's question is a very important one: Why should a host depress their immune system just when they need it most after major trauma, infection, or any insult of the type? This is why we are careful in separating the anergic elective *versus* intensive care unit patients, because we know that any insult such as trauma or infection will cause immunosuppression.

I do not know why this is happening, other than the host is trying to protect itself from self-antigen release by down-regulating the immune response. Teleologically, humans sustaining massive trauma with major bacterial contamination were not meant to survive. With less injury, such as breaking an ankle, which still releases self-antigens, the immune system may be down-regulated so that one does not react to these antigens and go into anaphylaxis.

What are the cells making *in vitro?* In the mixed lymphocyte culture reaction with allogeneic cells, or when T cells are stimulated with antigens or mitogens, they make several cytokines as long as you take these cells outside of the patient and treat them *in vitro.* They will behave just as anybody else's cells, whether they are anergic or reactive.

Again, the reduction in mortality that Dr. Mannick asked, in the last 5 years, I think has to do with improvements in survival practice. It is like the boy in a glass bubble in the case of severe combined immunodeficiency. If such patients are put in a sterile environment, they do not die of infection. I think this also addresses Dr. Baker's question.

Dr. Pruitt asked about the possible changes in microbial flora. I suppose we have seen some sort of switch. We see *Pseudomonas, Staphylococcus epidermidis,* and *Candida* in the surgical intensive care unit. What role this "switch" may play is not clear. There is a T cell-dependent host defense against *Pseudomonas,* for example. This has not been examined by us, but it may be playing a role in the surgical intensive care unit patients.