

DISCUSSION

DR. P. WILLIAM CURRERI (Mobile, Alabama): I would like to take this opportunity to thank Drs. Deitch and McDonald for inviting my discussion of their paper. Their work is extraordinarily exciting and provides new information that should prove helpful in describing the mechanisms responsible for immunological deficiency following major thermal injury.

As they have emphasized, the study of human cell-mediated immunity following major injuries may allow a rational use of immunomodulators to reduce the risk of sepsis. This approach offers more promise than the development of new antibacterial agents.

These investigators have presented data which suggest a marked elevation in the spontaneous blastogenic transformation (SBT) ratio above 20, associated with the onset of sepsis. In at least two patients, an abrupt decrease in the SBT ratio, accompanied by persistent clinical signs of sepsis, was indicative of imminent death. It appears that changes of spontaneous blastogenic activity were not related to the size of burn or patient mortality, but were closely associated with the incidence of septic episodes. Nevertheless, it is important to point out that the number of deaths in this series was small, and with greater patient experience and more sophisticated manipulation of data, patient mortality might be predicted by the rate of change of serial SBT ratios over a period of time.

I have several questions to address to the authors. It is stated that 60% of the patients who expired also had inhalation injuries. It might be important to know how many of the survivors had inhalation injury, and whether there was any correlation between SBT activity and pulmonary insufficiency. Is it possible that *in vivo* activation of suppressor cell activity or of cellular exhaustion or nonregulation of helper T-cells is a consequence of the site of injury—that is, the lungs—and, thus, may not be related to the magnitude of observable cutaneous injury?

I notice that the investigators used topical antimicrobial agents, consisting of either silver sulfadiazine or mafenide acetate. I wonder whether these topical agents were used in a random fashion, or whether all patients were initially treated with silver sulfadiazine until they developed septic complications of their wounds, at which time mafenide acetate was substituted. It has been shown that different topical agents have specific effects on both lymphocyte and leukocyte chemotaxis, and such topical agents may have unique effects on endogenously stimulated lymphocyte activity. In other words, is it possible that the increase of spontaneous lymphocyte activity is a reflection of the use of mafenide acetate, a more uncomfortable topical agent, during the intervals of life-threatening sepsis?

Finally, it should be emphasized that the authors have suggested that SBT increases are predictive of septic episodes. In this pilot study, they have presented data to substantiate this hypothesis. However, as I am certain the authors appreciate and recognize, proof of the value of increasing SBT as a predictor of sepsis will need a prospective study in which the investigators will need to predict sepsis on the basis of laboratory evaluation of spontaneous lymphocyte activity, which must then be correlated with specific measures of sepsis.

A disturbing aspect of this presentation is that the authors never outlined their definition of sepsis. Was this a clinical impression, or were specific, objective physiological measurements used to establish septic episodes?

I commend these authors for providing a new and original investigative study, which will clearly add to our growing informational base regarding post-thermal host resistance. I look forward to their continued studies of this patient population, which might provide a better laboratory tool for the quantification of sepsis, while at the same time providing an assay which will allow investigative evaluation of the efficacy of various techniques of immunomodulation.

DR. JOHN A. MANNICK (Boston, Massachusetts): I enjoyed this presentation and I congratulate Drs. McDonald and Deitch on their study.

I am absolutely convinced that they are correct that there is increased spontaneous blastogenic activity of the leukocytes in patients after burns and after a number of other kinds of injury, including, strangely enough, kidney transplantation. The question is: What is the meaning of this?

I certainly believe that the contribution they have made today is a worthwhile one; that is, an increase in this blastogenic activity is associated with septic episodes. However, I am not nearly so convinced that the spontaneous blastogenic activity is due to the activation of lymphocytes. The reason for this lies in the method of obtaining the leukocytes for study in these instances.

(Slide) The method shown here is universally used to obtain lymphocytes from peripheral blood. This is the Ficoll-Hypaque technique, designed as a sedimentation or floatation method of getting the lymphocytes away from red cells, platelets, and cells of the myeloid series; and one usually obtains, in blood from a normal individual, a preparation that is about 99% lymphocytes, with the occasional monocyte or macrophage.

(Slide) Shortly after burn injury, the population of cells obtained by the very same technique changes markedly, and as you can see here, there are all varieties of myeloid elements in this preparation of leukocytes from peripheral blood. Lymphocytes may even be a minority in the cell population after burn injury, and unless one is very careful to look at a stained sample like this, and take into consideration only lymphocytes, one has a hard time figuring out what the blastogenic activity means. The one mitogenic figure in this set seems to be a myeloblast.

No one has ever looked into this question in any thorough way in patients after burns, but in kidney transplant patients it has been reported that this blastogenic activity is almost exclusively due to proliferation of myeloid elements.

My question for Drs. McDonald and Deitch is: Have you got any autoradiographic evidence that the lymphocyte is actually the dividing element here? Perhaps all we are seeing is proliferation of myelocytic elements, particularly neutrophils and neutrophil precursors in response to burn injury. The increase in blastogenic activity may be nothing more arcane than this.

As to lymphocytes, (slide) whether they are lazy or overworked or just plain sitting around, I am not sure; but one thing is clear, and that is after a major burn, a burn of greater than 30% body surface area, the peripheral blood lymphocytes fail to produce a vital mediator molecule, interleukin 2, and this failure is very consistent after burn injury, and will persist in major burns as long as 50 to 60 days.

The reason I believe this is important (slide) is that interleukin 2 is a molecule that is necessary for the initiation of all varieties of cellular and humoral immune responses, and the failure to produce this molecule may be fundamental to the defects in host defenses seen after burn injury.

I enjoyed the paper very much, and I look forward to hearing Drs. McDonald or Deitch elaborate on the methodology used for preparing the lymphocyte preparations they studied.

DR. LOREN J. HUMPHREY (Shawnee Mission, Kansas): This is another fine paper by Dr. McDonald and his colleagues. Four or 5 years ago, Drs. Wood, Votenc, Mani, and I presented data on T-lymphocyte function in 27 patients with major thermal burns. We showed that immunosuppression relates to a decrease in the number of T-cells, rather than impaired function of the individual T-cell; although T-cell numbers remain depressed, in some patients the mitogen responses return to very high levels.

At that time, we thought that this might be due to a highly responsive T-cell population that was selected, or that there was a depletion of T-suppressor cells. I think Dr. Mannick has come upon one of the ideas; perhaps it is a lymphokine of some type. My question, Dr. McDonald, is: Do you have preliminary data—because, if I know him, he is already started on that—to find out the subsets of lymphocytes, and perhaps some of the immunoregulators?

DR. EDWIN A. DEITCH (Closing discussion): Let me first thank the discussants for their penetrating comments. Before I answer these questions, there are a couple of facts that we have to bear in mind. The first is that there is no such thing as one unique immunological defect that occurs in the stressed patient, trauma victim, or the postoperative patient that causes, or accounts for, the increased incidence of infections in these patients. What we are attempting to do in this study is to relate *in vitro* lymphocyte function to *in vivo* activity.

First, to answer Dr. Curren's questions. There was no statistical difference between the incidence of inhalation injuries in those patients who survived *versus* those patients who died. Also, no relationship existed between the SBT and various other factors, including the burn size, third degree component, or location of the burn; however, some of the p values were provocative (that is, $p = 0.07$, $p = 0.10$). Perhaps, as more patients are accrued, there will be a statistical relationship between SBT and burn size.

Silvadene® was used initially, and Sulfamylon® was only used when the patient became septic or resistant to Silvadene. We do not believe that our results are due to the effect of these topical agents on cell function, especially since the data that has been published on topical agents shows inhibition of cellular activity, rather than accentuation of cellular activity. Sepsis was defined as a wound culture with greater than 10^5 organisms and/or positive blood cultures with systemic signs of infection—ileus, hypotension, and hyperglycemia. Patients who had no systemic signs, regardless of what was cultured, were not considered septic.

Dr. Mannick has raised a very critical question: Exactly what cells are we studying? The answer to this question is not known. The mechanisms by which we and other investigators isolate these mononuclear cells are relatively crude. We attempted to get a better handle on this problem by taking some of these cells and putting them through a flow cytometer after labeling them with the monoclonal antibodies OKT-4, OKT-8, and OKT-3. The number of cells in the burn patients which did not label with OKT-4, OKT-8, or OKT-3 was slightly higher than in the controls. This represented only about 10%

of the cell population. These cells may be immature mononuclear cells or cells of the myeloid series.

We attempted to decipher what is going on *in vivo*. (Slide) We were interested in looking at the effect of the burn environment on the cellular function, of not just the lymphocyte, but also the neutrophil. Therefore, we measured neutrophil oxygen consumption. Interestingly, changes in neutrophil metabolism were similar to that documented in the lymphocyte. That is, the neutrophil's endogenous activity was increased, stimulated activity was decreased, while the total oxygen consumption was normal. What this really says is that, in many ways, the metabolism of the cell is not much different from the metabolism of the burn patient that is, after the burn, the metabolic rate increases, especially during sepsis.

(Slide) This last slide illustrates the lymphokine activity produced by the patient's cells. We collected the supernatants from both stimulated and unstimulated lymphocytes after 24, 48, and 72 hours in culture. The key point of this slide is that there is no difference between lymphokine activity of control *versus* patient cells. It is of interest that both the patients' cells and the control cells, which had not been stimulated, supported the maximum blastogenesis of the subsequent stimulation with PHA better than the supernatants of cells which had been stimulated. This indicates, or at least suggests, that an activated cell may be producing soluble factors (lymphokines) that prevent subsequent *in vitro* activation; therefore, what we may be seeing is a manifestation of down-regulation or the production of suppressor substances.