Discussion

DR. NICHOLAS L. TILNEY (Boston, Massachusetts): I was delighted to have the opportunity to discuss this paper from this particular group, who have been working on these curious dendritic cells for several years now.

As we have heard, acute allograft rejection is mediated primarily by T lymphocytes. Once activated by graft antigen, many of these cells develop the capacity to elaborate lymphokines, active effector molecules.

In contrast, dendritic cells have no recognized effector function but appear to be specialized immunostimulatory cells that can activate resting T lymphocytes.

Theoretically, by removing such cells from the graft, as we have heard, an important trigger for rejection can be blocked, with resultant graft prolongation.

If dendritic cells are so critical, my first question is: Why is graft prolongation not more reproducible in various transplant models from which such cells in the grafts have been removed or destroyed?

Dendritic cells in normal or transplanted animals migrate from tissue to tissue, apparently via blood stream and lymph, a physiologic process that may amplify sensitization of the host against antigenic stimuli.

In this paper Dr. Larsen and his colleagues distinguish nicely between donor and recipient dendritic cells. An obvious criticism, however, of any dendritic cell work is that there is no specific marker for these cells, and they can only be identified by negative selection. That is, all other identifiable cell populations are removed, leaving behind only putative dendritic cells, which are then stained. The obvious question, of course, is: Are these really dendritic cells?

The investigators have also shown that dendritic cells migrate from graft to spleen, an observation at variance with the usual dogma that suggests that sensitization of host cells occurs within the graft itself and not within lymphoid tissues of the host.

If this migration pathway to spleen is so important, why are the results of recipient splenectomy so variable, extending graft survival in some models but not in others?

This slide shows isologous mouse spleen dendritic cells, the blue spots, which migrate to normal splenic white pulp. To emphasize how effete this cell population is, it takes about ten mouse spleens at about 100 million cells apiece to produce a million of these blue dendritic cells.

In this paper, the authors have shown differences between cells such as these dendritic leukocytes and donor cells migrating from heart graft to spleen. Do these two dendritic cell populations have differential migration patterns? Specifically, do they go to different anatomic compartments in the white pulp of the spleen?

And finally the question of phenotype arises. As we have heard, Langerhans cells, maturing in culture, change their phenotype. Do you have any evidence that maturing cardiac dendritic cells do the same?

DR. MARK A. HARDY (New York, New York): I have shared with the Oxford group an interest in dendritic cells and particularly their migration and homing, not only because this is important for the understanding of antigen recognition and of mechanisms of rejection but also for the practical reason that this may offer approach to prolonging allografts without toxic immunosuppression of the host, perhaps even by manipulation of the donor organ itself.

I do, however, have some questions that are in many ways similar to Dr. Tilney's comments and questions.

We also have shown in the past that the donor organ, the cardiac allograft, quickly depletes itself of donor-type dendritic cells that go to the spleen. We do not quite understand, however, why these dendritic cells do not also go to lymph nodes, and they in fact do go there sometimes, particularly when a splenectomy is done. I wonder if the authors could comment on whether there is any special attraction about the spleen and whether there are chemotactic factors that attract these dendritic cells or antigen-presenting cells, which may be a better name for these cells, to the spleen, rather than to the lymph nodes.

The other question is, why doesn't the recipient antigen presenting cell go to the graft where there are already T cells with the appropriate receptors? Why don't the recipient dendritic cells go to the donor graft and offer a different pathway of transmission of information, an alternate pathway, if you will?

And finally, I would like to have Dr. Larsen comment about peripheral sensitizations, even though he emphasizes the centralization concept, as we have in our work over the past many years. How does he explain the findings of others in terms of peripheral sensitization using the sponge matrix models and other models, where most of the sensitization may occur, in fact, in the donor graft?

Is there a relationship between the central sensitization and the peripheral sensitization, particularly in regard to the antigen presenting cells, and is that correlation important regarding timing?

DR. ROBERT J. CORRY (Iowa City, Iowa): During the mid-1970s we were involved in protocols of donor pretreatment with cytoxan and steroids to eliminate passenger leukocytes with very little improvement in graft survival, if any, and I am wondering if we should reconsider those efforts.

You and others have shown that suppressor cells are produced in the spleen. Does the antigen-presenting cell have any role in creation of the suppressor cells?

The other new, novel Japanese drug, 15-deoxyspergualin, seems to affect the antigen-presenting cell, and I am wondering if you have any plans to use that drug experimentally.

DR. JOSHUA MILLER (Miami, Florida): The paradox in transplantation has always been with the antigen-presenting cell being of donor origin, whereas in all other immune responses the antigen-presenting cell is of recipient origin, where the co-recognition of the MHC class 2 by the helper T cell is always in what is called a restrictive pattern. That is, the recipient has to recognize recipient (autologous) MHC.

In my mind this has always been a paradox, and I would like to know if in your paper you have dealt with this paradox. Have you looked at—this is to amplify Mark Hardy's point—the recipient antigen-presenting cells? What happens to them in the spleen in relationship to the donor APCs?

DR. CHRISTIAN P. LARSEN (Closing discussion): First I would like to address Dr. Tilney's question as to why we consider the Ia^+ leukocytes of heart to be dendritic leukocytes (DL). Work on DL has been hampered by the lack of DL-specific monoclonal antibodies, which stain DL of nonlymphoid tissues. However, we have presented evidence that the DL of heart are phenotypically extremely similar to the Langerhans cell of skin (the DL of the epidermis). In addition, we have shown with two-color immunofluorescence that the cardiac DL do not express B-cell, T-cell, and some conventional macrophage markers (F4/80).

In addition, as Dr. Tilney mentioned, classic splenic dendritic cells do not display effector functions, which distinguishes them from macrophages. Similarly Hart and Fabre have shown that the DL of heart are nonphagocytic. In the studies reported here, we have isolated the DL from heart and found that, like classic dendritic cells, these cells possess immunostimulatory activity, that is, the ability to initiate a primary T-cell response. Therefore the DL of heart are phenotypically and functionally very similar to dendritic cells from lymphoid tissues.

Next I would like to address why depletion of donor DL from grafts prior to transplantation does not consistently lead to long-term graft survival. In many animal models if one can remove the donor DL prior to transplantation, prolonged graft survival can be obtained. However, this is not uniformly the case. One reason for these inconsistencies was suggested by Derek Hart, who found that in order to achieve an effect, 95% of the donor DL had to be depleted. So, one answer is that in some studies there may not have been adequate DL depletion. However, this is clearly not the whole answer, because there have been very carefully performed studies where near complete DL depletion has been achieved, and yet the grafts are still rejected.

This leads us to the question of whether or not host DL are involved in rejection. There is limited *in vitro* and *in vivo* evidence that in some cases that host DL can process and present graft antigens to host T cells and initiate an anti-donor response. However it appears from the depletion studies that in most cases that presentation by host cells is a weaker route of sensitization, hence our interest in donor DL.

The question regarding the role of splenectomy is particularly pertinent in light of the migration of donor DL into the spleen. Splenectomy has been investigated both experimentally and clinically in prospective ran-

domized trials. In many rat transplantation models splenectomy results in prolonged graft survival, and in man renal allograft survival was improved at 2 years in splenectomized patients. In both cases, however, it is clear that splenectomy alone does not prevent sensitization.

There are at least two possible explanations for this observation. DL can leave tissues via the lymph, as well as via the blood. Therefore, it seems likely that the donor DL migrate not only to the spleen, but also to the lymph nodes, as lymphatic connections are re-established. There, they might sensitize recipient T cells. Alternatively, host DL in lymph nodes may be involved in sensitization.

Dr. Tilney has noted that there are subtle differences in the localization of DL within the spleen in the present study and a previous study from our group (J Exp Med 1988; 167:646–651). In the earlier study, syngeneic splenic dendritic cells localized primarily in the central white pulp, whereas in this study the allogeneic cardiac DL localized in the peripheral white pulp. At present we do not have an explanation for this interesting difference. One possibility is that donor DL enter the spleen at the marginal zone. During their migration toward the central white pulp they may encounter alloreactive T cells that arrest further migration.

Dr. Hardy noted that dendritic cells fail to migrate into lymph nodes from the blood. This was shown in the studies by Drs. Austyn and Kupiec-Weglinski. From the blood, dendritic cells migrate exclusively into the spleen, the entry into spleen being T-cell dependent. When DL are injected into nude, T-cell–deficient mice, they fail to home to the spleen, but if the animals are reconstituted with T cells, the DL home to the spleen as in normal animals. So it appears that T cells within tissues might be able to alter the endothelium to promote recruitment of dendritic cells.

This point also relates to question of whether recipient DL migrate into allografts. We have investigated this question using ^{111}Indium-labeled host-strain dendritic cells. We found that mature DC did not migrate into cardiac allografts in the early postoperative period after transplantation. In this setting, even though T cells were present in the grafts, host DC were not recruited.

In the long term, if an allograft is accepted, there is evidence that recipient DC do eventually repopulate grafts. Based on our data, we would predict that during the generation of immune responses, such as allograft rejection, that they too would migrate centrally.

Finally Dr. Hardy has noted that, in contrast to our data, studies using sponge-matrix allografts have suggested that site of sensitization is primarily peripheral, in the graft. There may be important difference between sponge matrix grafts, which are often prepared with donor splenocytes, and vascularized organ allografts that contain nonlymphoid DL, akin to Langerhans cells. As we discuss in the manuscript, DL in the periphery cannot initiate immune responses unless they are stimulated by the cytokine GMSCF, whereas splenic DC are mature immunostimulatory cells. Therefore sponge-matrix allografts may contain a different population of DL than those normally present in allografts.