The Use of Allopeptides in Tolerance Induction in Rodents

Mark A. Hardy, O. O. Oluwole, H. A. DePaz, R. Gopinathan, M. Garrovillo, S. F. Oluwole and A. O. Ali

Graft 2002; 5; 91
DOI: 10.1177/152216280200500207

The Use of Allopeptides in Tolerance Induction in Rodents

Mark A. Hardy, O. O. Oluwole, H. A. DePaz, R. Gopinathan, A. O. Ali, M. Garrovillo, and S. F. Oluwole

The development of new approaches to the induction of transplant tolerance has remained the Holy Grail in the field of organ transplantation. The following summarizes the use of major histocompatibility complex (MHC) allopeptides in the induction of tolerance to various cellular and solid organ allografts in a fully MHC mismatched model. Initial studies using host myeloid dendritic cells, primed with donor allopeptides injected intrathymically or intravenously, led to indefinite cardiac allograft survival. Although the intrathymic route of administration is clinically impractical, it has dominated experimental methods of tolerance induction over the past several years. We have presently developed the use of intravenous administration of either dendritic cells primed in vitro or T-cells primed in vivo with donor MHC Class I immunodominant allopeptides to induce permanent organ allograft acceptance. The mechanism of allogeneic unresponsiveness in this model appears to be highly dependent on the short-term presence of the thymus since ablation of the thymus within the 1st week of grafting abrogates allograft prolongation. Subsequent cell migration studies showed that activated host T-cells recirculate to the thymus, emphasizing the importance of indirect allopresentation in tolerance induction.

Introduction

It is well known that the thymus plays a major role in the development of self-tolerance and may play a role in acquired tolerance in transplantation. Having previously shown that intrathymic (i.t.) injection of resting donor T-cells (major histocompatibility complex [MHC] Class I) induces tolerance to subsequently transplanted organ allografts in the rat, we have recently shown that i.t. injection of a single immunodominant alloMHC Wistar-Furth (WF) class I (RT1.A^{u}) peptide 5 (P5), when combined with 0.5 ml of ALS, also induces acquired systemic tolerance. On the basis of the hypothesis that host thymic dendritic cells play a major role in induction of such tolerance, we further demonstrated that host thymic dendritic cells pulsed with P5 in vitro injected i.t. with 0.5 ml of ALS i.p. also induced tolerance to organ allografts in the fully MHC-mismatched WF to ACI rat combination.[1,2] On the basis of these findings, we were encouraged to test the possibility that peripheral intravenous (i.v.) inoculation of alloMHC class I peptide-primed host antigen presenting cells (DCs) may be equally effective and provide us with a clinically relevant model of tolerance induction.

Activated Myeloid Dendritic Cells and Their Role in the Induction of Unresponsiveness to Organ Allografts

With improvement in DC isolation and culture techniques, large numbers of DCs can be induced from bone marrow cells and propagated for therapeutic purposes. We have therefore embarked on the study of the use of host DC derived from bone marrow following stimulation with GMSF and IL-4, as a carrier of processed-specific target antigen (specific class I immunogenic allopeptide) to T-cells to induce immunologic unresponsiveness to organ and cell allografts. Our initial thought was that

Mark A. Hardy, M.D.
Department of Surgery
Columbia University College of P&S
161 Ft. Washington Ave.
New York, New York, USA 10032
email: mah1@columbia.edu

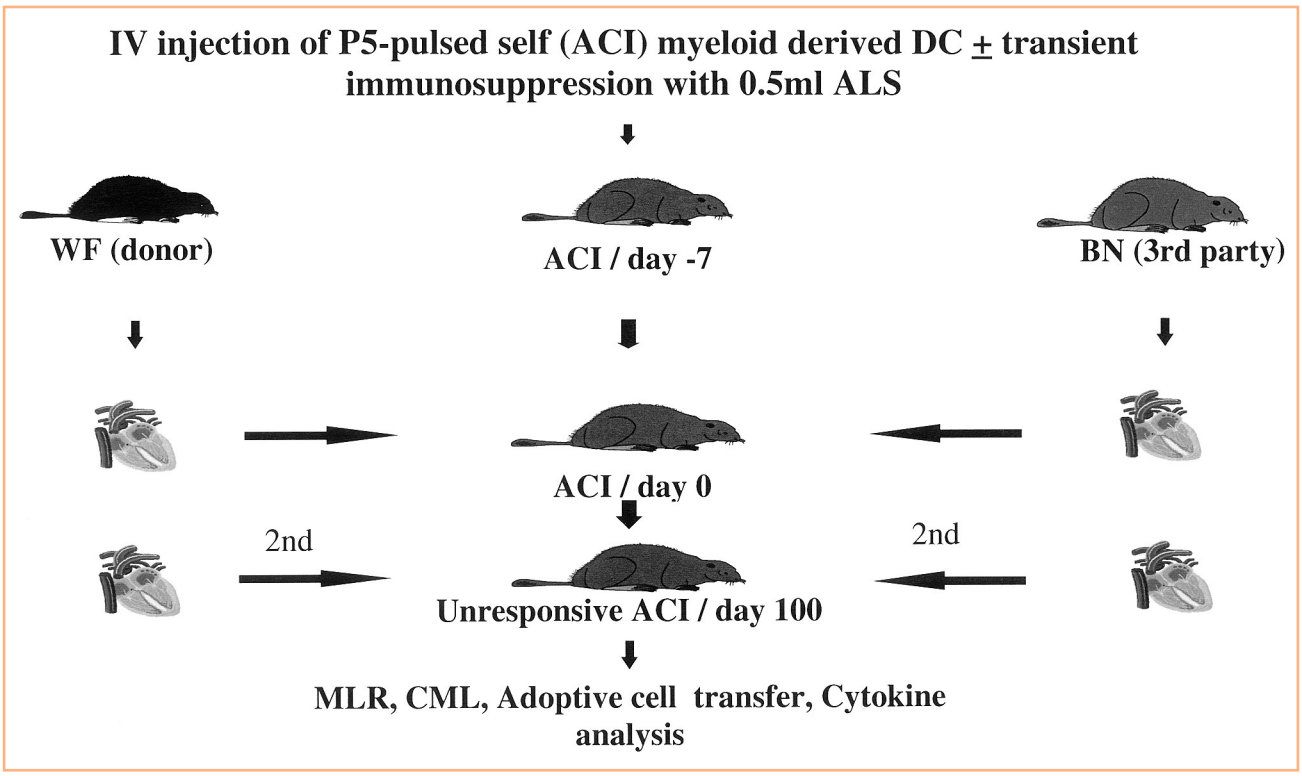

Figure 1. Experimental protocol.

DCs circulate through the thymus and initiate deletion of alloreactive thymic T-cells.

In the 1st series of experiments, myeloid or thymic DCs were pulsed in vitro with P5 (WF) and subsequently injected either i.t. or i.v. into ACI recipients with 0.5 ml of ALS i.p. (Fig. 1). The animals were transplanted 7 days later with either WF or 3rd-party (BN) cardiac allografts. We were again able to show that i.v. injection of P5-primed host thymic DCs led to cardiac allograft unresponsiveness to WF allografts, with 100% donor-specific permanent ACI cardiac allograft acceptance. Long-term unresponsive recipients accepted permanently donor-specific 2nd-set cardiac and islet allografts but not skin allografts. Similar results were obtained when, instead of recipient's thymic DCs, recipient's bone marrow cells were stimulated to differentiate into DCs in vitro and subsequently pulsed with P5. ACI recipients injected i.v. with P5-pulsed myeloid host DC (with 0.5 ml ALS) on day –7 showed cardiac allograft survival and 2nd-set donor-specific allograft acceptance identical to recipients pretreated with P5-pulsed thymic DCs. This effect was abrogated by recipient's thymectomy within the 1st 1 to 3 weeks following transplantation, but not subsequently. This suggests that "intrathymic education" is required for the induction of tolerance in this model. Based on our previous theory that thymic DC uptake, process, and present the i.t. inoculated allopeptide to developing T-cells during the induction of acquired thymic tolerance, we concluded at this point that alloMHC peptide-pulsed DCs might recirculate to the host thymus and induce antigen-specific T-cell tolerance via indirect allorecognition. To explore this point, we proceeded with the next series of experiments.

Migration Patterns of Activated T-Cells

We now examined the likely possibility that i.v.-injected, P5-pulsed host DCs induce tolerance by recirculation to the thymus; we also considered the

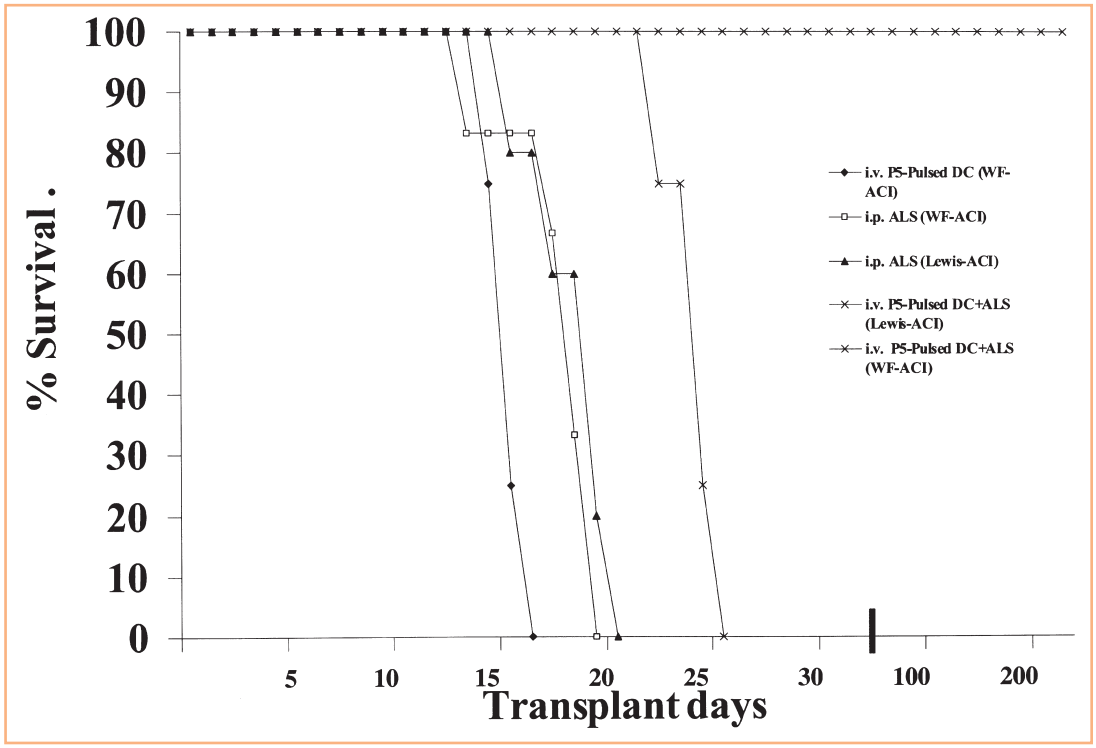

Figure 2. Effect of i.v. inoculation of P5-pulsed DC on cardiac allograft survival.

possibility that the peptide-alloreactive T-cells generated in vivo after the i.v. injection of P5-pulsed DCs may provide the critical signal in the thymus that leads to deletion of alloreactive T-cells. To study these possibilities, in vivo P5-activated syngeneic splenic T-cells were obtained from ACI rats pretreated with i.v. injection of P5-pulsed host DCs 7 days prior to harvesting the T-cells. At the same time, we cultured myeloid DCs in vitro with GM-CSF and IL-4 stimulation prior to priming them with P5. Naive and P5-pulsed myeloid or thymic DCs, and in vivo P5-primed T-cells, were labeled with ^{111}Indium oxine (^{111}In) as we have previously described.[3] ^{111}In labeled DC (5×10^6) or P5-primed T-cells (2×10^7) were injected intravenously into syngeneic rats and subsequently sacrificed serially at 3, 24, 48, and 72 h, to determine the percentage of injected radioactivity per organ (%ID/organ). As expected, the labeled naive myeloid or thymic DCs were immediately sequestered in the lung before migrating to the liver and spleen; the DCs did not accumulate in the peripheral lymph nodes (0.01% ID). Labeled P5-pulsed syngeneic DCs were found not to enter or reenter the native thymus. Therefore, we concluded that circulation of P5-pulsed DCs to the host thymus is not the underlying mechanism of tolerance in our model. Since i.v. injection of P5-pulsed host DCs induces P5-specific alloreactive T-cells in the host, it appeared possible that recirculation of in vivo alloreactive T-cells to the thymus may be responsible for tolerance induction. Using naive-labeled T-cells for the kinetic studies, we showed that they home to the liver, spleen, and lymph nodes but do not reenter the thymus.[4] In contrast, in vivo–activated T-cells do recirculate from blood to the native thymus for up to 72 h in relatively large numbers. We therefore could conclude that antigen-specific selected alloreactive peripheral T-cells (by ALS preconditioning or by other undefined means) play an important role in the induction, and possible maintenance, of acquired systemic tolerance in this model via their continuous recirculation to the thymus. It appears that the thymus is open to

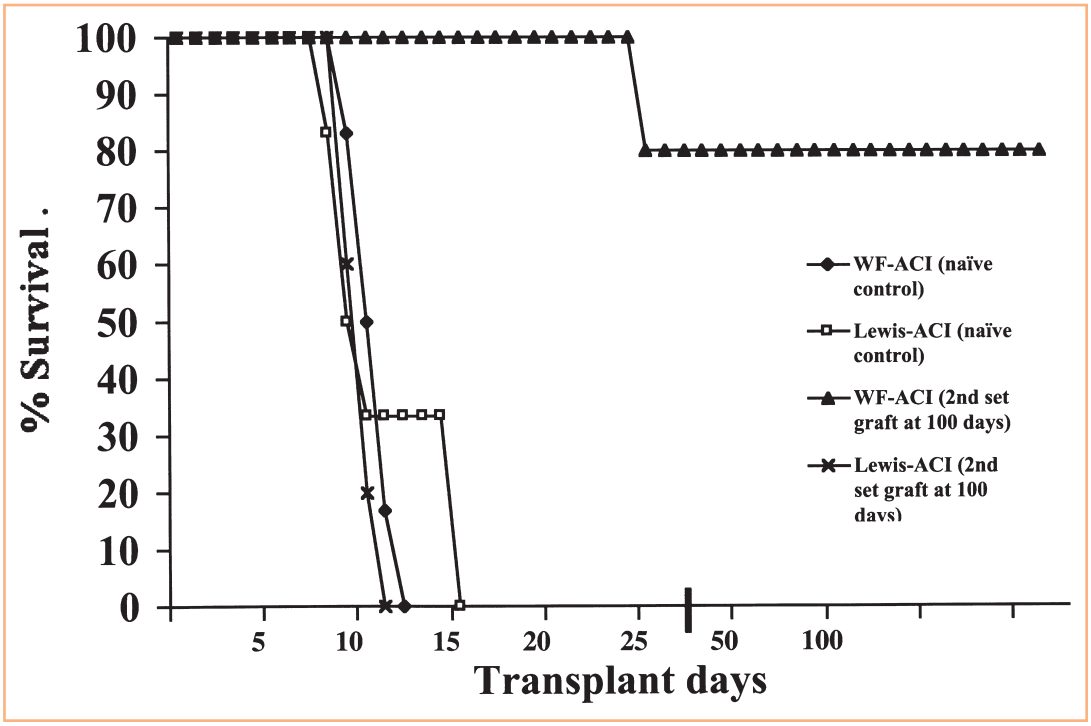

Figure 3. Survival of 2nd-set cardiac allograft in long-term unresponsive recipient.

2-way traffic with the periphery and may function as the initiator of tolerance induction and, therefore, may be one of the sites important for tolerance induction.

The Role of Adoptive Transfer of Activated T-Cells in Tolerance Induction

Parallel to these studies, we examined the functional role of the alloreactive T-cells from recipients that previously received P5-pulsed host DCs. Seven days after the syngeneic myeloid DCs were administered by the i.v. route into ACI rats, the splenic T-cells shown by MLR to be primed to P5 were harvested and then injected either by i.t. or i.v. route into syngeneic naive ACI recipients of donor (WF) or 3rd-party (Lewis) cardiac allografts. The syngeneic splenic T-cells (2×10^7) that showed a high MLR proliferative response to P5 in context of self MHC were able to significantly prolong WF cardiac allografts (18.6 ± 1.8 days versus 10.5 ± 1.0 days in controls). Donor-specific permanent graft survival (> 200 days) resulted when 0.5 ml ALS was added to this treatment on day –7. Thymectomy performed before i.v. injection of the alloreactive T-cells abrogated such tolerance induction. This confirms that homing of in vivo–activated T-cells to the host thymus plays an important role in tolerance induction. It was not surprising therefore that i.t. injection of P5-primed T-cells also resulted in donor-specific permanent graft survival, subsequent (> 200 days) 2nd-set donor-specific cardiac allograft survival, and normal rejection of 3rd-party allografts in the controls.

Summary and Conclusion

In these studies, we demonstrate that allogeneic class I MHC allopeptides can be effectively processed in vitro by recipient dendritic cells obtained from bone marrow after appropriate stimulation. Such allopeptides processed in vitro by syngeneic DCs activate T-cells through the indirect allorecognition pathway. Such alloreactive T-cells are able to induce donor-specific tolerance in adoptive transfer experiments and have now been shown to recirculate to the host thymus. We can also conclude that the presence of the host thymus is neces-

sary for a minimum of 1 to 3 weeks following allografting.[5] There is also a 1-week interval required between intravenous injection of P5-pulsed self DCs or alloreactive T-cells prior to allografting to induce tolerance to the organ.

Although recirculation of primed T-cells to the thymus appears to be necessary in our model, there remains the possibility that intrathymic emigrants, which are susceptible to being recruited into a regulatory pool, reinforce the regulatory ability of the primed T-cells. The need for injection of 1 dose of ALS also raises the possibility that this agent partially selects the T-cell population in the recipient to favor immunoregulatory T-cells. Yet it remains uncertain what may be the relative importance of the state of maturity of the dendritic cells obtained from the recipient's bone marrow in relation to tolerance induction. The role of alloreactive T-cells, and their interaction with thymic DCs, appears to be critical in tolerance induction in this model. Cloning of such immunoregulatory T-cells offers obvious possibilities, which we are now intensively studying with excellent preliminary results. Immunogenic allopeptides are available in both large animals and man and offer the potential for inducing immunologic unresponsiveness in the host without the use of chronic immunosuppression. These studies offer new and promising possibilities of clinical application, and preclinical studies in swine have been initiated.

References

1. Garrovillo M, Ali A, DePaz HA, Gopinathan R, Oluwole OO, Hardy MA, et al. Regulatory role of thymic dendritic cells (DC) in acquired thymic tolerance: induction of tolerance to cardiac allografts by adoptive transfer of allopeptide-pulsed host thymic DC. **Transplant Proc 2001;33:149**.
2. Garrovillo M, Ali A, DePaz HA, Gopinathan R, Oluwole OO, Hardy MA, et al. Induction of transplant tolerance with immunodominant allopeptide-pulsed host lymphoid and myeloid dendritic cells. **Am J Transplant 2001;1:129-37**.
3. Gopinathan R, DePaz HA, Oluwole OO, Ali AO, Garrovillo M, Fawwaz RA, et al. Mechanisms of acquired tolerance induced by adoptive transfer of alloMHC peptide activated T-cells: migration of effector T-cells to the thymus to induce T-cell tolerance. **Surg Forum 2000;51:322-4**.
4. Agus DB, Surh CD, Sprent J. Re-entry of T cells to the adult thymus is restricted to activated T cells. **J Exp Med 1991;173:1039-46**.
5. Ali A, Garrovillo M, Oluwole OO, Gopinathan R, DePaz HA, Jin MX, et al. Mechanisms of acquired thymic tolerance: induction of transplant tolerance by intrathymic injection of in vivo allopeptide primed host T cells. **Transplantation 2000;69:S394**.