Published in final edited form as:

Nat Med. 1998 September ; 4(9): 1006–1008. doi:10.1038/1993.

The art of tolerance

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In a mouse skin transplant model in which donor and recipient mice differ by only one minor histocompatibility antigen, graft tolerance can be induced up to ten days after transplantation, but endures only if there is persistence of migratory antigen (pages 1015–1019).

The connection between allograft rejection and the adaptive immune reaction to microorganisms was unmasked in 1973 with the discovery by Zinkernagel and Doherty¹ of the major histocompatibility complex-restricted host immune response to the lymphocyte choriomeningitis virus (LCMV) and to other non-cytopathic microorganisms. However, the means by which allografts escape rejection has remained one of the most controversial questions in biology ever since the description of neonatal tolerance in mice, which is strongly associated with donor leukocyte chimerism².

Workers in Zinkernagel's laboratory have taken a fresh look at this enigma using experimental models that place viral and transplantation immunology on common ground. Using transgenic techniques, they have produced colonies of animals that express transgenes encoding well characterized viral epitopes. With this technology it has been possible to pool the vast reservoir of information in viral immunology (particularly that acquired for LCMV) with the repository of knowledge in transplantation immunology, and to address controversial questions about transplantation tolerance^{3–5}.

The transgenic donor mice used in the experiments of Ehl *et al.*³, reported on page 1015 of this issue, ubiquitously express the gp33 peptide of the LCMV glycoprotein. When cells or tissues from these mice are infused or transplanted into unaltered mice of the same strain, the gp33 epitope constitutes a single minor histocompatibility class I-restricted antigen. This results in rejection of the transgenic skin by normal mice of the same strain in 70–120 days. In a subgroup of experiments, the recipient effector system was 'pre-armed' by insertion of a T-cell receptor transgene that increases the frequency of naive gp33-specific precursor cytotoxic T lymphocytes (CTL). The time to rejection of the gp33-positive skin grafts was reduced to 15 days. These investigators demonstrated that there is an absolutely specific relationship between the gp33 peptide and its cognate host CTL clone.

In their new experiments, the authors convincingly show that tolerance to the gp33 peptide expressed by the transgenic donor skin grafts can be reliably induced by conditioning the normal recipients in two ways: with three injections of a synthetic gp33 peptide (which is eliminated by the unaltered mouse in about 60 days) or with a single injection of long-lived spleen cells expressing gp33. They assess the relative effectiveness of tolerance inducing protocols through their ability to prolong survival of the gp33-positive skin grafts. Survival results were compared with data on the frequency of gp33-specific CTL precursor cells. Both the short-lived synthetic gp33 and the gp33-expressing spleen cells were tolerogenic, whether given before or up to ten days after skin transplantation. However, the full development and perpetuation of the tolerogenic effect depended on the persistence of gp33 expressed by donor spleen cells (that is, it depends on donor splenocyte chimerism).

Tolerance is not sustained by the transgenic skin grafts alone in the animals in which original non-reactivity was induced with synthetic gp33 peptide.

The principles defined in this exceptionally pure model can be readily identified in the more complex setting of clinical transplantation in which two populations of immune competent cells confront each other under an umbrella of immunosuppression. However, in contrast to the simplicity and precision of the Ehl model, four interrelated events, all regulated by antigen migration and localization, must occur in relatively close temporal proximity for organ allografts to be accepted by patients and in most experimental models⁵⁻⁸ (see figure). These are: (1) antigen-specific Clonal exhaustion/deletion of the recipient immune reaction; (2) reciprocal exhaustion of the allograft immune competent cells (passenger leukocytes) that migrate to host lymphoid organs after transplantation; (3) maintenance of the exhaustion of (1) and (2); and (4) reduction of the organ's antigenicity by progressive depletion of its passenger leukocytes, making the allograft an object of increasing immune indifference to the host immune system.

The first three of these events are dependent not only on the persistence of antigen, but also on its transport to the milieu of organized lymphoid tissue where growth factors, different cell types, and other factors necessary for efficient immune activation are located⁵. The short-lived synthetic gp33 peptide administered to recipient mice after transplantation in the Ehl experiments has been previously shown by the authors to reach these destinations⁹. However, with bioelimination of the synthetic peptide, tolerance cannot be maintained by the outlying transgenic skin graft, even though the histocompatibility barrier is a weak one (that is, skin graft survival for 70–120 days in unmanipulated recipients). Similarly, the disappearance of microchimerism in an organ recipient presages loss of the outlying allograft due to chronic or acute rejection^{8,10}. In the Ehl model, skin graft rejection was associated with thymus-dependent recovery of precursor CTL.

The tolerance induced by the gp33 expressed in the presumably self renewing mobile spleen cells is enduring and resistant to tolerance breakdown maneuvers, even though the chimeric cells can no longer be identified by flow cytometry in the blood after 60 days. Terakura *et al.*¹¹ have shown in a rodent allograft model that a large proportion of allogeneic leukocytes surviving the early post-transplant period leave the blood and lymphoid organs and find niches in non-lymphoid tissues and organs of the host, such as skin and heart.

Such secondary relocation, a phenomenon also seen with widespread non-cytopathic pathogens¹², would explain the findings of Ehl *et al.* and also why blood samples and lymphoid organ biopsies cannot be used to reliably monitor the presence or level of chimerism in organ allograft recipients. Periodic leakage of chimeric cells from the non-lymphoid to the lymphoid compartment after transplantation has been suggested as an explanation for the maintenance of clonal exhaustion^{5,8}. This is comparable to the stable equilibrium between destructive and non-destructive immunity described in an analogous model of autoimmune diabetes mellitus¹³.

Finally, the information provided by the Ehl experiments adds to the evidence that giving adjunct donor leukocyte infusions to organ recipients is an attempt (that can be safely delayed) to augment the normal chain of post-transplantation tolerogenic events. Under the circumstances of conventional organ transplantation, these tolerogenic events depend on the migration of passenger leukocytes from the donor allografts⁶⁻⁸. Although it is disappointing to note that the reported tolerogenic protocols used by the authors could not override the degree of pre-sensitization induced by pretransplant immunization with LCMV, tolerance has been shown by others¹⁴ to be produced in presensitized mice by the large bolus of

hepatic passenger leukocytes released into the recipient circulation after liver transplantation.

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1. . Figure 1

The figure depicts the four events that occur in close temporal approximation when there is successful organ engraftment: double acute clonal exhaustion and subsequent maintenance clonal exhaustion (1, 2, and 3 above) plus loss of organ immunogenicity due to depletion of the graft's passenger leukocytes (4, below).