sup; daf-2(e1370) hermaphrodites at the non-permissive temperature and examining cross progeny for dauer formation. The descendants of the cross progeny were also examined to ensure that the mutations were not unlinked, noncomplementing mutations. We also mapped many of the mutations by testing for linkage with unc-29, which maps near daf-16, or else with restriction fragment length polymorphisms using PCR (32).

15. One such gene may be daf-18. This gene has been identified by a single mutation that suppresses both dauer formation and the life-span extension of daf-2 mutants (7, 10, 28). However, many daf-18 individuals showed severe morphological abnormalities, suggesting that this gene has other, possibly essential functions (29). The fact that we did not find any daf-18 alleles supports this hypothesis. In addition, we note that because we screened F1 progeny of mutated animals, we would have missed mutations that were maternally recessive.

16. We first attempted to clone daf-16 by positional mapping but found that the gene was located in a gap in the physical map between cosmid AE7 and ZK98. To isolate daf-16:Tc1 insertion mutants, we screened daf-2 (as198); mut-6 animals for spontaneous dauer mutants that did not become dauer worms when cultured at 20°C. One mutant, mu417, also suppressed dauer formation at 25°C (10). This mutation failed to complement daf-16(m28) and was closely linked to unc-29, which maps near daf-16. mu417 was subsequently crossed to either unc-29(e1072), daf-2(e1370); hm5(e1480) or daf-2(e1370); hm5(e1490) mutants, and homozygous Daf-16(−) and Daf-16(+) recombinants were obtained. Genomic DNA was prepared from these recombinants and analyzed by Southern blot hybridization with the 5.1-kb Tc1 sequence as a probe. A 6.1-kb Tc1-hybridizing fragment was detected in the Xba I–digested genomic DNA, which was present in 20 of 20 daf-16(−) recombinants but absent in 15 of 15 daf-16(+) recombinants and also absent in the wild-type strain (N2). DNA from the corresponding region was then extracted from agarose gels and purified by serial digestion with Eco RV. The remaining sequence was then used as a probe in subsequent experiments.


21. K. Lin, J. Dorman, C. Kenyon, data not shown.


30. Neither gain of function (n1046) nor dominant negative (s100) mutations in the C. elegans Fas homolog let-60 affected C. elegans life-span (because these mutants cannot lay eggs, their gonads were ablated to prevent premature death from internal bacterial infection). In addition, let-60(n1046) did not suppress the dauer-constitutive phenotype of daf-2(e1370) (J. A. Eppel and C. Kenyon, unpublished data).


37. We thank N. Ahmada for technical assistance with positional mapping (16); B. Albinnder, M. Macrae, J. Reiter, T. Wang, D. Eisenstadt, I. Reichardt, and J. Blumstein for helping to isolate and characterize tri-methylpsoralen- and Tc1-induced daf-16 mutants; J. A. Eppel in our laboratory for investigating the role of Ras in the daf-2 signaling pathways (30); members of the Kenyon lab for discussions and comments on the manuscript; and Y. Kohara for producing EST clones of daf-16. Supported by NIH grant AG18116. C.K. is the Herbert Boyer Professor of Biochemistry and Biophysics.

38. J. Apfeld in our laboratory for investigating the role of Ras in the daf-2 signaling pathways (30); members of the Kenyon lab for discussions and comments on the manuscript; and Y. Kohara for producing EST clones of daf-16. Supported by NIH grant AG18116. C.K. is the Herbert Boyer Professor of Biochemistry and Biophysics.
able to generate FasL-expressing myoblasts that persisted as differentiated multinucleated myotubes for more than 80 days in vivo. Moreover, our data show that islet destruction is not prevented but accelerated by FasL-expressing myoblasts, presumably through a bystander effect mediated by infiltrating neutrophils.

Our findings are in direct conflict with those reported by Lau et al. (1). Subtle differences can perhaps be invoked; however, it is unclear from our studies how myoblasts that express both Fas and FasL can avoid apoptosis while differentiating or be available to induce apoptosis of invading lymphocytes, as proposed by Lau et al. Even if clones of non-Fas–expressing myoblasts were selected by Lau et al. (1) and were therefore spared from apoptosis, it remains an enigma how such FasL-expressing myoblasts escaped the granulocytic response that we observed and which resulted in premature elimination of both Fas-deficient myoblasts (lpr) and islets. It has been suggested that the exact quantity of FasL expressed may be critical in determining whether immunoprotection or immunodestruction occurs (12). However, we and others have found that, although low amounts of FasL expression do not result in granulocytic infiltration, these amounts also do not protect against T cell–mediated allograft rejection (6, 13). FasL-specific activity is known to vary as a result of polymorphisms (14); however, both Lau et al. (1) and we used the C57BL/6 form of FasL known to have reduced cytotoxic potential. Taken together, these findings suggest that, although FasL may have a role along with other factors in the immune privilege of the eye and testis, expression of endogenous FasL alone is unlikely to suffice.

In support of this conclusion, most cell types and tissues that have been genetically engineered to express FasL have been shown to undergo destruction by neutrophils (5, 6). Thus, FasL expression has complex consequences (15), and further investigation of the effects of dosage, cell context, and microenvironment are warranted. Our observations, although discouraging for transplant purposes, suggest other applications for FasL, and new approaches for defining the molecular determinants requisite for immune protection.
REFERENCES AND NOTES

11. S.-M. Kang et al., data not shown.
15. D. R. Green and C. F. Ware, ibid., p. 5986.
16. We thank S. Nagata for supplying cDNA for Southern blotting probes; C. Goodnow for supplying lpr mice; Z. Lin, P. Kraft, and N. Braeoci for technical assistance; and N. Asher, A. Estelles, and D. Vaux for helpful discussion. Supported by a Howard Hughes Medical Institute Physician Research Fellowship to S. M.K., a postdoctoral fellowship from the Deutsche Forschungsgemeinschaft to A.H., an NSRA (F32 HL08991) to M.L.S., and grants from NIH (R01CA59717, R01-HD18179), and MERIT award R37 AG09621 to H.M.B.

Response: Kang et al. raise several issues with regard to engineered expression of FasL on myoblasts as a means of providing immunoprotection to islet allografts. In our initial studies, we observed the expression of FasL on myoblasts from C57BL/6 mice cultured over a long term; when differentiated in vitro, these cells did not undergo apoptosis and continued to express functional FasL. In contrast, Kang et al. report that FasL expression of C3H myoblasts results in apoptosis after differentiation. It is unclear why FasL expression differs among different myoblast populations; perhaps apoptosis resistance is acquired during multiple passage, or susceptibility to FasL-induced apoptosis may be strain dependent. We have transfected nonobese diabetic (NOD) mice myoblasts and observed spontaneous cell death; however, when myoblasts were preselected with prolonged culture with soluble FasL, we obtained apoptosis-resistant cells that permitted subsequent functional expression of FasL. Preliminary co-transplantation experiments with allogeneic islets with these NOD FasL+ myoblasts have not resulted in prolonged survival. This cell mortality may be a result of the greater complexity in the killing of primed T cells that infiltrate and destroy the islets, as one would expect to find in the diabetic NOD recipient.

With regard to the issue of neutrophil infiltration, we have reexamined the histology of the composite grafts from our initial study on day 3 after transplantation and have observed local inflammation, but islets and myoblast were present. Histology at day 7 revealed local pockets of neutrophilic infiltration, but again, islets and myoblast were identified. By the fifth week after transplantation (as we originally observed [1]), there was resolution of the inflammation, and fused muscle cells were seen on histology. The prolongation of islet allograft survival we observed appears to be a bystander effect of local expression of FasL in which there is no specificity in the killing of infiltrating T cells. Thus, there may be a race between the muscle cells and islets to survive the initial inflammation and still effect apoptosis of infiltrating activated T cells directed against the allogeneic islets. Under such circumstances, transplantation of borderline numbers of islets required for correction of hyperglycemia would not result in long-term correction of the diabetic state, because the initial inflammation would result in some attrition of islets. In this regard, as noted in our initial studies [1], we observed fluctuation in glucose in diabetic mice receiving the highest numbers of FasL expressing myoblast (2 x 10^6). In retrospect, this may have represented the initial inflammation, which subsided 3 weeks after transplantation, as reflected in stabilization of glucose in the blood [1]. The amount of FasL expression may be critical in this regard in that there may be a balance between FasL induced local inflammation and immunoprotection [2].

We agree that immune privilege is more than the expression of FasL and that there are other factors at work, especially in light of our inability to extend these findings to the NOD mouse model of spontaneous diabetes. However, our initial studies showing that even a bystander effect can prolong islet allograft survival [1] suggest that expression of FasL in the context of alloantigen or autoantigen (as in the case of islets) may enable specific killing of T cells that are activated toward such coexpressed antigens. Although muscle cell expression of FasL in a local fashion may not be applicable across all strains or species (because of self apoptosis or destruction by neutrophils in a confined space), ectopic FasL expression (in the context of an alloantigen or autoantigen on engineered cells such as muscle) administered systemically may effect specific attenuation of an immune response. This attenuation has been demonstrated by Arai et al. with the use of an allogeneic tumor cell engineered to express FasL [3]. These recent findings may help define a role for the use of engineered FasL expression in the modulation of immune response.

Henry T. Lau
Department of Pediatric Surgery,
Johns Hopkins Hospital,
Baltimore, MD 21287-3716, USA
C. J. Stoeckert
Department of Hematology,
Children’s Hospital of Philadelphia,
Philadelphia, PA 19104, USA

REFERENCES


19 September 1997; accepted 22 October 1997