port is calculated, backward transport across a bedform crest line is subtracted from the forward transport. Therefore, it is treated mathematically as "undoing" the bedform despite the fact that such transport must help create the bedform. In contrast, when gross transport is calculated, transports in both directions across the crest line are summed, or treated as creating the bedform.

In natural flows, where sediment transport can occur toward any number of directions, the most convenient technique for predicting bedform trend is to compute for the given flow conditions the gross bedform–normal transport for a variety of arbitrary bedform trends (for example, at 1° increments), and to select the trend that yields the maximum value. With this technique we can take into account the transport vectors for the 3 or 4 seconds during which the experimental sediment surface was turned between stationary positions, and it can be shown that our experimental values of ϕ should be approximately 5° greater than if the turning were instantaneous. In addition to explaining the small systematic discrepancy between the observed and predicted values of ϕ, the sand transport during turning explains why the break in bedform behavior was observed to occur at a divergence angle between 90° and 112°, rather than exactly at 90°.

This experimental study does not consider some known causes of bedform obliquity, such as along-crest variations in the rate of bedform migration resulting from variations in local flow conditions (*19, 20*). Also, the results do not prove that other controls, such as sediment availability, flow strength, or helical vortices, have no effect on bedform trend. However, the results do demonstrate that longitudinal and oblique bedforms can be produced independently of such controls. We would expect plots that are qualitatively similar to Fig. 2 to describe the orientation of such natural bedforms as eolian dunes, tidal sand waves, and combined current-wave ripples, provided that the bedforms are large enough (relative to the amount of sediment transported during each flow cycle) to respond only slightly to the individual flow cycles. These natural bedforms are known to be approximately transverse to the resultant transport direction in flows that are effectively unidirectional (corresponding to flows in which either the divergence angle is small or the transport ratio is large). Transverse bedforms are also known to occur where the divergence angle is approximately 180° and the transport ratio is greater than 1.0. In addition, tidal sand waves, oscillation ripples, and eolian dunes will trend parallel to the resultant transport direction when the transport ratio is 1.0 and the divergence angle approaches but does not equal 180°. All of these bedforms of known behavior are in agreement with the trends that were observed in the experiments and predicted from the rule of maximum gross bedform–normal transport. Quantitatively, the relations for different bedforms in different fluids might differ from those in Fig. 2. For example, the abrupt transition from transverse to longitudinal trends that occurs when the divergence angle exceeds 90° might occur at different angles, possibly because of flow separation, which occurs to a different extent over different kinds of bedforms. Possibilities for future work include experimental study of bedform trend in directionally varying subaqueous flows and field studies of larger natural bedforms in flows that produced the bedforms.

In summary, the experimental bedforms had trends that yielded the maximum gross bedform–normal transport. The fact that transverse, oblique, and longitudinal bedforms all followed the same rule of alignment suggests that they do not require differing flow dynamics for their origin (that is, they are basically the same kind of bedform). Longitudinal bedforms were created without flow parallel to the bedform crest line and without alignment with preexisting paired helical vortices in the flow. No bedforms were aligned parallel to the resultant transport direction unless such a trend yielded the maximum gross bedform–normal transport. This study demonstrates that bedforms can trend at any angle relative to the resultant transport direction and that an infinite number of flow regimes can produce the same bedform trend. Until future studies devise techniques for distinguishing along-crest and across-crest components of sediment transport, it will not be possible to determine transport directions from remote-sensing images of dunes in deserts or sand waves on the sea floor; bedforms can no longer be presumed to trend parallel or normal to the resultant transport direction.

REFERENCES AND NOTES

1. R. A. Bagnold, *Geogr. J.* **78**, 13 (1933).
2. K. W. Glennie, *Desert Sedimentary Environments* (Elsevier, Amsterdam, 1970).
3. C. R. Twidale, *Erde* **112**, 231 (1981).
4. J. A. Mabbutt, R. A. Wooding, J. N. Jennings, *Aust. J. Sci.* **32**, 159 (1969).
5. A. Warren, *Nature (London)* **259**, 653 (1976).
6. R. A. Brown, in *Eolian Sediments and Processes,* M. E. Brookfield and T. S. Ahlbrandt, Eds. (Elsevier, Amsterdam, 1983), pp. 291–310.
7. H. Tsoar, *Sedimentology* **30**, 567 (1983).
8. N. Lancaster, in (*6*), pp. 261–289.
9. S. G. Fryberger, *U.S. Geol. Surv. Prof. Pap. 1052* (1979), pp. 137–169.
10. R. E. Hunter, B. M. Richmond, T. R. Alpha, *Geol. Soc. Am. Bull.* **94**, 1450 (1983).
11. R. A. Bagnold, *The Physics of Blown Sand and Desert Dunes* (Methuen, London, 1941).
12. M. Mainguet and M.-C. Chemin, in (*6*), pp. 353–363.
13. R. J. Wasson and R. Hyde, *Nature (London)* **304**, 337 (1983).
14. The analysis in (*13*) was disputed by D. M. Rubin [*ibid.* **309**, 91 (1984)].
15. S. R. Hanna, *J. Appl. Meteorol.* **8**, 874 (1969).
16. G. Tseo, thesis, University of Adelaide, Adelaide, Australia (1986).
17. R. L. Folk, *Sedimentology* **23**, 649 (1976).
18. A. Warren and P. Knott, in (*6*), pp. 343–352.
19. D. M. Rubin and R. E. Hunter, *Sedimentology* **32**, 147 (1985).
20. W. R. Dietrich and J. D. Smith, *Water Resour. Res.* **20**, 1355 (1984).

21. We thank D. Drake and B. Jaffe at the U.S. Geological Survey, Menlo Park, CA, for their reviews of the manuscript.

19 December 1986; accepted 15 April 1987

Islet Allograft Survival After a Single Course of Treatment of Recipient with Antibody to L3T4

JUDITH A. SHIZURU, ANITA K. GREGORY, C. TIEN-BAO CHAO, C. GARRISON FATHMAN

Allografts of pancreatic islets of Langerhans were induced to survive for an indefinite period in diabetic mice if, at the time of engraftment, the mice received a single course of treatment with a monoclonal antibody directed against the L3T4 determinant, a nonpolymorphic cell surface glycoprotein present on the cell surface of the murine T helper-inducer lymphocyte subset. This treatment allowed the survival of islets of Langerhans transplanted across a major histocompatibility barrier without additional immunosuppression. The results demonstrate that the lymphocyte subset defined by the expression of the L3T4 molecules is central to the induction of allograft rejection and provides a model for tolerance induction for organ allograft transplantation.

T CELLS SERVE BOTH REGULATORY and effector functions during allograft responses (*1–3*). Although T lymphocytes play a central role in the rejection of allografted tissue (*4–6*), the relative importance of the different T cell populations in graft rejection remains controversial

Division of Immunology, Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305.

(*7–9*). Recent studies have suggested that the murine T cell helper-inducer subset, characterized by cell surface expression of the L3T4 molecule, is necessary in the initiation of both humoral and cellular immune responses (*10–20*). The lymphocyte subset defined by the expression of the L3T4 molecule ($L3T4^+$) correlates with "restricted" recognition of class II major histocompatibility complex (MHC) products on antigen-presenting cells (*10, 11*). Antibodies against L3T4 used in vitro block antigen-specific functions of MHC class II–restricted T cells (*11*). Monoclonal antibodies directed against L3T4 have been successfully used in vivo to suppress the humoral immune response against soluble antigens (*13–16*) and to block the expression or perpetuation of autoimmune disease in murine models of experimental encephalomyelitis (*17*), systemic lupus erythematosus (*18*), and collagen-induced arthritis (*19*). In addition, prolongation of skin grafts has been demonstrated by treatment of recipient mice with antibody to L3T4 (*20*).

In the complex series of events that lead to first-set allograft rejection, the $L3T4^+$ T lymphocyte population has been recognized as being important in the initiation but not in the effector phase of graft rejection (*3, 8, 9*). The necessity of the helper-inducer subpopulations during actual rejection, however, is still a widely debated issue (*7, 8*). In this report, we demonstrate that administration of a monoclonal antibody to L3T4 at the time of engraftment can allow indefinite survival of pancreatic islet allografts in diabetic mice.

The monoclonal antibody used in these studies, GK1.5, is a rat immunoglobulin (IgG2b) directed against mouse L3T4 (*11*). We showed earlier that intraperitoneal administration of GK1.5 (100 μg given on each of three consecutive days) caused selective long-term depletion of more than 90 percent of the $L3T4^+$ T cells (*15*). Complete elimination of the L3T4 subset, however, was not achieved at even very high doses of GK1.5 (*13–16*). Five percent of the $L3T4^+$ cells persisted in the spleen and lymph nodes, and treatment with GK1.5 did not reduce the number of $L3T4^+$ thymocytes. In the present study, we gave a total of 350 μg of GK1.5 to diabetic mice (100 μg on days −1, 0, and +1, and 50 μg on day +2 relative to islet engraftment) at the time they received allogeneic islet grafts. The mice did not receive any subsequent immunosuppressive therapy.

A chemical diabetes was induced in C57BL/6 ($H-2^b$) mice by the administration of a single intravenous bolus (200 mg/kg) of streptozotocin (*21*). An animal was considered to be a suitable recipient when its plasma glucose exceeded 450 mg/dl on two consecutive bleedings at least 3 days apart. In control animals that did not receive transplants, no spontaneous remission from the streptozotocin-induced diabetes was noted at any time during the study. Islets of Langerhans isolated from adult A/J ($H-2^a$) mice by collagenase digestion of the pancreas (*22*) were transplanted into the livers of recipient diabetic C57BL/6 (B6) mice (*23, 24*). The response of control untreated diabetic B6 animals treated with allogeneic A/J islets was a transient drop in the hyperglycemia immediately after the transplantation, followed by a rise in the plasma glucose values several days later indicating the onset of rejection (*24*) (Fig. 1A). These untreated control diabetic animals that received allografts were not given exogenous insulin; they had accelerated weight loss and died several months after rejection of the islet allografts.

In contrast, B6 mice that were given antibody GK1.5 at the time they received allogeneic A/J islets showed indefinite acceptance of their allogeneic islet grafts as evidenced by persistent normoglycemia. In all ten of the diabetic B6 mice that were reconstituted with 600 to 800 A/J islets, allograft survival (normoglycemia) persisted beyond 90 days (Fig. 1B). Earlier studies have shown that streptozotocin causes diabetes by two different mechanisms (*25, 26*). A large bolus (200 mg/kg) (the amount used in these studies) has direct toxic effects on the insulin-secreting beta cells, with no evidence of lymphocyte infiltration, and results in a swift onset of overt diabetes (*21*). A low dose regimen (40 mg/kg, five times a day) causes pronounced lymphocytic infiltration (*24, 25*) of islets, and hyperglycemia appears over the course of several weeks.

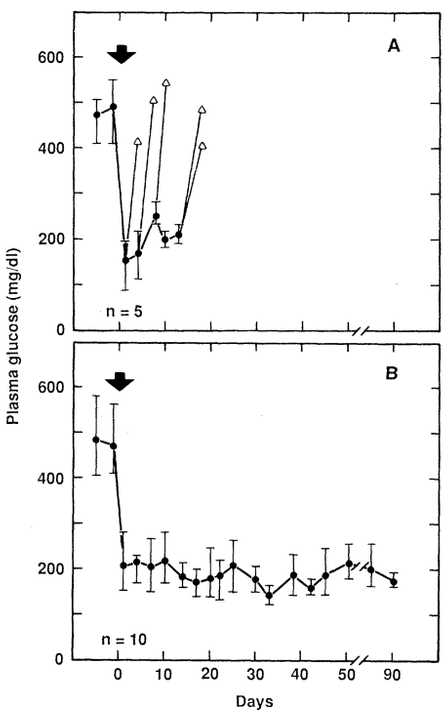

Fig. 1. Suppression of islet allograft rejection with monoclonal antibody GK1.5, which is directed against the L3T4 molecule. Plasma glucose levels after allogeneic islet reconstitution are shown for (**A**) control untreated C57BL/6 diabetic mice transplanted with allogeneic islets and (**B**) for GK1.5-treated C57BL/6 diabetic mice transplanted with allogeneic islets. Closed circles are mean plasma glucose values ± standard deviation. Open arrows (➔) represent day of rejection (rise is plasma glucose) for an individual transplant recipient. Bold arrows (➡) define day of islet transplantation.

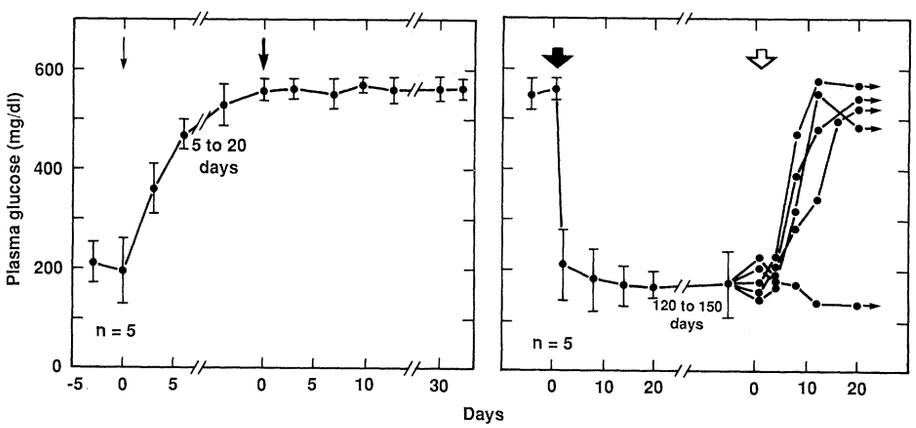

Fig. 2 (**left**). Plasma glucose levels of streptozotocin-induced diabetic C57BL/6 mice that received a course of monoclonal antibody GK1.5 only. The left half of the figure shows the increase in plasma glucose following streptozotocin administration (➔). In these animals, approximately 5 days after intravenous administration of streptozotocin, plasma glucose values (in nonfasting animals) rose from normal levels (150 to 250 mg/dl) to more than 450 mg/dl. Diabetic mice were given a 4-day treatment with GK1.5 (➔) as in Fig. 1 but did not receive islet grafts. Diabetes was not reversed in any of these animals. **Fig. 3** (**right**). Plasma glucose levels of streptozotocin-induced diabetic C57BL/6 mice that received a course of treatment with monoclonal antibody GK1.5 at time 0 (➡); 120 to 150 days later, during transplant-induced normoglycemia, the mice were challenged with 5×10^7 donor syngeneic (A/J) irradiated (3300 R) (⇨) spleen cells. Rejection is indicated by hyperglycemia appearing 5 to 10 days after this injection with allogeneic cells.

Because GK1.5 effectively ameliorates various autoimmune syndromes, we administered the antibody in an identical manner to a control group of mice made diabetic by a large bolus of streptozotocin (but not receiving transplants) to investigate the possibility that we were treating an autoimmune component of streptozotocin-induced diabetes (*17–19*). None of the animals in this group showed signs of recovery (Fig. 2).

Earlier studies had shown that long-term stable endocrine allografts could be rejected if large numbers of splenocytes syngeneic to the allograft were administered to the recipient (*24*). Thus recipient B6 mice that had maintained their allografts for more than 150 days after GK1.5 treatment were challenged with donor-specific allogeneic A/J spleen cells in an attempt to cause rejection of the allograft. The dose of spleen cells used to cause rejection was titrated and, as demonstrated in Fig. 3, "tolerance" could be broken, and long-standing islet grafts were rejected in four of five animals when 5×10^7 A/J spleen cells were administered intraperitoneally. Thus, continued long-term unresponsiveness is not due to the deletion of antigen-reactive effector lymphocytes or to the lack of target alloantigens on the islet grafts.

To further understand the mechanism (or mechanisms) by which treatment with antibody to L3T4 allowed the long-term survivors to maintain their allografts, we considered the possibility that a persistent "hole in the T cell repertoire" after regeneration of the T helper subpopulation might result in a subsequent inability to recognize the alloantigens on the islet allografts. Lymph node cells of naïve mice contain a large proportion of precursor T cells capable of responding to MHC alloantigens (approximately 1:20) (*27*). Therefore, if holes in the T helper repertoire existed, syngeneic B6 lymph node cells from naïve recipients should provide replacement for allorecognition of the "tolerated" A/J allografts. When long-term allograft survivors were given intravenous injections of 10^5 to 10^7 syngeneic (B6) lymph node lymphocytes from naïve recipients, there was no ensuing graft rejection.

Preliminary adoptive transfer studies suggest that active suppression may be responsible for the long-term allograft acceptance. However, the underlying mechanism of graft acceptance is not established. These experiments demonstrate that transient removal of the helper-inducer T cell subset can lead to indefinite allograft survival and underscore the importance of this subset during tissue rejection. Such studies suggest a rationale for similar tolerance induction studies in primates and may lead to the application of this mode of immunotherapy in man.

REFERENCES AND NOTES

1. B. M. Hall and S. E. Dorsch, *Immunol. Rev.* 77, 31 (1984).
2. T. B. Strom, J. W. Jupler-Weglinski, C. D. Heideck, N. L. Tilney, *Transplant. Proc.* 17, 801 (1985).
3. D. W. Mason and P. J. Morris, *Annu. Rev. Immunol.* 4, 119 (1986).
4. J. F. A. P. Miller, *Proc. R. Soc. London Ser. B* **156**, 415 (1962).
5. M. J. Dallman, D. W. Mason, M. Webb, *Eur. J. Immunol.* 12, 511 (1982).
6. A. E. Chang and P. H. Sugarbaker, *Transplantation* 29, 381 (1980).
7. K. J. Lafferty, *Transplant. Proc.* 17, 1549 (1985).
8. S. J. Burakoff, *ibid.*, p. 1546.
9. D. Steinmuller, *Transplantation* 40, 229 (1985).
10. P. Marrack, *J. Exp. Med.* 158, 1077 (1983).
11. D. P. Dialynas, *Immunol. Rev.* 74, 29 (1983).
12. A. M. Krensky and C. Clayberger, *Transplantation* 39, 339 (1985).
13. R. Benjamin and H. Waldmann, *Nature (London)* 320, 449 (1986).
14. R. Benjamin, S. P. Cobbold, M. R. Clark, H. Waldmann, *J. Exp. Med.* 163, 1539 (1986).
15. J. Goronzy *et al.*, *ibid.* **164**, 911 (1986).
16. D. Wofsy, D. C. Mayes, J. Woodcock, W. E. Seaman, *J. Immunol.* 135, 1698 (1985).
17. M. K. Waldor *et al.*, *Science* 227, 415 (1985).
18. D. Wofsy and W. E. Seaman, *J. Exp. Med.* **161**, 378 (1985).
19. G. E. Ranges, S. Sriram, S. M. Cooper, *ibid.* **162**, 1105 (1985).
20. S. P. Cobbold, A. Jayasuriya, A. Nash, T. D. Prospero, H. Waldmann, *Nature (London)* **312**, 548 (1984).
21. G. Brosky and J. Logothetopoulos, *Diabetes* **18**, 606 (1969).
22. P. E. Lacy and M. Kostianovsky, *ibid.* **16**, 35 (1967).
23. P. E. Lacy, J. M. Davie, E. H. Finke, *ibid.* **30**, 285 (1981).
24. D. Faustman, V. Hauptfeld, P. Lacy, J. Davie, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5156 (1981).
25. A. A. Like and A. A. Rossini, *Science* **193**, 415 (1976).
26. Y. T. Kim and C. Steinberg, *Diabetes* **33**, 771 (1984).
27. M. Simonsen, *Cold Spring Harbor Symp. Quant. Biol.* **32**, 317 (1967).

28. We thank M. Casey for excellent technical assistance and D. Bet and R. Kizer for their expertise in assembling this manuscript. Supported by Juvenile Diabetes Foundation fellowship grant 386279 and NIH grants DK32075 and DK37104.

9 February 1987; accepted 20 May 1987

Tissue Distribution and Developmental Expression of the Messenger RNA Encoding Angiogenin

HENRY L. WEINER, LYNNE H. WEINER, JUDITH L. SWAIN

New blood vessel growth occurs during normal fetal development and in diseases such as cancer and diabetes. The polypeptide angiogenin induces new blood vessel growth in two biological assays and may play a role in the vascular development of the fetus and in the neovascularization that accompanies diseases and wound healing. A complementary DNA probe for human angiogenin was used to examine the tissue distribution of angiogenin messenger RNA (mRNA) in the developing rat and in selected transformed cell lines. Angiogenin mRNA was detected predominantly in adult liver but was also detectable at low levels in other tissues. The expression of the angiogenin gene in rat liver was found to be developmentally regulated; mRNA levels were low in the developing fetus, increased in the neonate, and maximal in the adult. The amount of angiogenin mRNA in human HT-29 colon carcinoma and SK-HEP hepatoma cells was not greater than that in normal rat liver. These results demonstrate that angiogenin is predominantly expressed in adult liver, that the pattern of angiogenin gene expression is not temporally related to vascular development in the rat, and that the transformed cells studied do not contain more angiogenin mRNA than does normal liver. If angiogenin activity is controlled at the transcriptional level, the results of this study suggest that the primary function of angiogenin in vivo may be in processes other than the regulation of vascular growth.

ANGIOGENIN, A POLYPEPTIDE WITH a molecular size of 14 kD, was originally isolated from conditioned media of the HT-29 human adenocarcinoma cell line (*1*). Angiogenin is an inducer of vascular growth in the chick chorioallantoic membrane and the rabbit cornea and has been suggested to be a mediator of vascular development (*2*). At the amino acid level, angiogenin has significant homology with pancreatic ribonuclease and catalyzes the cleavage of 28*S* and 18*S* ribosomal RNA (*3*). The significance of the ribonuclease activity of angiogenin is unclear. Angiogenin contains a signal peptide characteristic of secreted proteins, but the site of synthesis, the stimulus for secretion, and the site of action of angiogenin are unknown. The human angiogenin gene has been cloned and sequenced (*4*), but the tissue distribution and developmental regulation of angiogenin gene expression have not been described. This report details an analysis of

Department of Medicine, Duke University Medical Center, Durham, NC 27710.