the receptor under nondenaturing conditions is estimated to be 1000 kD, about four times the apparent mass of the dissociated chains under denaturing conditions.

26. Supported by grants from NIH (GM24032 and GM30387 to L.S. and AI22449 to D.H.). T.M. was a Swiss National Science Foundation Fellow. We thank B. Baird for stimulating discussions, T. Wensel for the Ca^{2+} chelating column, and P. Majerus for inositol 1:2-cyclic 4,5-trisphosphate.

2 February 1988; accepted 1 April 1988

ADP-Ribosyltransferase Activity of Pertussis Toxin and Immunomodulation by *Bordetella pertussis*

W. J. BLACK,* J. J. MUNOZ, M. G. PEACOCK, P. A. SCHAD, J. L. COWELL, J. J. BURCHALL, M. LIM, A. KENT, L. STEINMAN, S. FALKOW

Pertussis toxin is produced by the causative agent of whooping cough, *Bordetella pertussis*, and is an adenosine diphosphate (ADP)–ribosyltransferase capable of covalently modifying and thereby inactivating many eukaryotic G proteins involved in cellular metabolism. The toxin is a principal determinant of virulence in whooping cough and is a primary candidate for an acellular pertussis vaccine, yet it is unclear whether the ADP-ribosyltransferase activity is required for both pathogenic and immunoprotective activities. A *B. pertussis* strain that produced an assembled pertussis holotoxin with only 1 percent of the ADP-ribosyltransferase activity of the native toxin was constructed and was found to be deficient in pathogenic activities associated with *B. pertussis* including induction of leukocytosis, potentiation of anaphylaxis, and stimulation of histamine sensitivity. Moreover, this mutant strain failed to function as an adjuvant and was less effective in protecting mice from intracerebral challenge infection. These data suggest that the ADP-ribosyltransferase activity is necessary for both pathogenicity and optimum immunoprotection. These findings bear directly on the design of a nontoxic pertussis vaccine.

PERTUSSIS TOXIN IS THE PRIMARY determinant of virulence produced by *Bordetella pertussis* in whooping cough (*1–3*). Aspects of the systemic pathology of the disease, including lymphocytosis and hypoglycemia, can be reproduced in laboratory animals with purified toxin alone (*4*). The toxin is composed of five dissimilar polypeptides that can be divided into two functional subunits (*5*); an "A" monomer, S1, mediates adenosine diphosphate (ADP)–ribosylation of host G proteins (*6*), and a "B" oligomer, composed of four different polypeptides, designated S2 through S5, mediates binding of the toxin to host tissue (*7*). Two molecular mechanisms of pathogenesis have been proposed for pertussis toxin. The first is the ADP-ribosylation and concomitant inactivation of host G proteins involved in normal eukaryotic cell metabolism (*6*). The second mechanism is the lectin-like binding of the B oligomer to eukaryotic cells (*7*), which has been proposed to act mitogenically to cause the lymphocytosis and other immunomodulatory activities mediated by pertussis toxin (*8*).

Pertussis toxin is also found in, and is considered to be a primary protective component of, both the traditional whole-cell (*2, 9*) and the newer acellular (*10*) formulations of the pertussis vaccine. However, there is speculation that active toxin present in the vaccines may cause certain rare but serious vaccination sequelae including hypotonic, hyporesponsive syndrome, convulsions, and encephalopathy (*11*). Recent efforts to clone the toxin genes (*12*) are in part predicated on the proposition that an enzymatically inactive version of the toxin molecule produced by modified toxin genes might serve as a valuable component in a defined vaccine. We were interested in determining the contribution of the ADP-ribosyltransferase activity to pathogenesis and immunoprotection and so constructed *B. pertussis* strains with defined mutations in the toxin genes. These genes were assayed for the induction of leukocytosis (*4, 13*), the potentiation of anaphylaxis (*4, 14, 15*), and the stimulation of histamine sensitivity (*4*). We also examined the capacity of the strains to serve as adjuvants (*4*) and their immunoprotective activity against experimental *B. pertussis* infection in mice (*16*).

A *B. pertussis* strain with a nonpolar mutation that altered the primary structure of the pertussis toxin S1 or ADP-ribosyltransferase subunit was constructed by in vitro linker scanning mutagenesis (*17*), followed by allelic exchange (*18, 19*) of the mutation into the *B. pertussis* chromosome. This mutation, *ptx*A3201, introduced a 12-bp insertion at the Sal I restriction site of the S1 gene (Fig. 1), maintaining the reading frame integrity and introducing four novel codons, for Val-Asp-Gly-Ser, between Tyr^{141} and Val^{142} (*12*). We chose this site for modification because of its proximity to Glu^{140}; Collier and co-workers have shown that for each of two other ADP-ribosyltransferase toxins, diphtheria toxin and pseudomonas exopro-

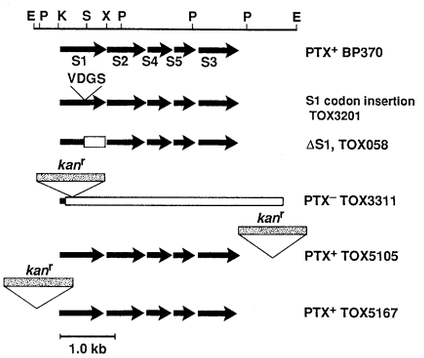

Fig. 1. Pertussis toxin operon mutations. Defined mutations in the pertussis toxin operon were constructed in vitro by means of standard recombinant DNA technology (*30*) and introduced into the chromosome of *B. pertussis* strain BP370 (*18*) by allelic exchange (*18, 19*). The parental *B. pertussis* strain, PTX^+, BP370, contains a polycistronic arrangement of the genes for the five toxin polypeptide subunits (*12, 18*). The S1 codon insertion derivative, TOX3201, contains a 12-bp insertion, GACGGATCCGTC, at the Sal I site in the S1 gene, introducing the amino acids Val-Asp-Gly-Ser into the S1 polypeptide between Tyr^{141} and Val^{142} (*12*). The ΔS1 derivative, TOX058, contains a deletion of the 3′ half of the S1 gene, from the Sal I site to the Xba I site, fusing the S1 codon for Asp^{143} to the stop codon in the Xba I site. The construction of TOX3201 (*19*) and TOX058 (*27*) is described in greater detail elsewhere. TOX3201 was previously designated BP370*ptx*-3201 (*19*). The PTX^- derivative, TOX3311, has a deletion extending from about 200 bp inside the 5′ end of the S1 gene down through about 1100 bp 3′ of the S3 gene, and a kan^r gene (*26*) ligated into the breach (*18*). The PTX^+, TOX5105 derivative has an insertion of the kan^r gene about 800 bp 3′ of the toxin structural genes (*18*). The PTX^+, TOX5167 derivative has an insertion of the kan^r gene about 400 bp 5′ of the toxin structural genes (*18*).

W. J. Black and S. Falkow, Department of Medical Microbiology, Stanford University, Stanford, CA 94305.
J. J. Munoz and M. G. Peacock, Laboratory of Pathobiology, NIAID Rocky Mountain Laboratories, Hamilton, MT 59840.
P. A. Schad and J. L. Cowell, Department of Bacteriology, Praxis Biologics, Inc., Rochester, NY 14623.
J. J. Burchall, Burroughs Wellcome Company, Research Triangle Park, NC 27514.
M. Lim, A. Kent, L. Steinman, Department of Pediatrics, Stanford University, Stanford, CA 94305.

*To whom correspondence should be addressed at Department of Microbiology, Emory University School of Medicine, Atlanta, GA 30322.

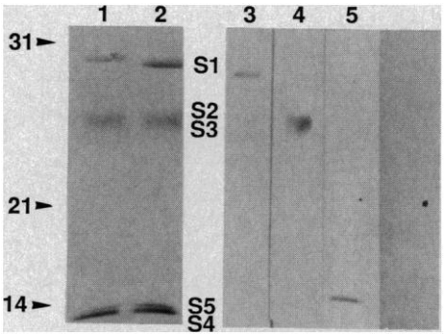

Fig. 2. TOX3201 export and assembly. Pertussis toxins were purified from culture supernatants of strains BP370 and TOX3201 by fetuin-Sepharose affinity chromatography (*22*). On analysis by SDS–polyacrylamide gel electrophoresis (*23*) and Coomassie blue staining, both the TOX3201 (lane 1) and the BP370 (lane 2) toxins exhibited all of the subunits of a complete holotoxin structure (*5, 22*). Western immunoblot (*24*) analysis of the TOX3201 toxin showed reactivity with monoclonal antibodies to the S1 (lane 3), S2 (lane 4), and S4 (lane 5) subunits of pertussis toxin. Molecular mass standards are indicated by arrows and are given in kilodaltons.

tein A, a Glu residue is a critical component of the enzymatic active sites (*20*). Chou and Fasman analysis (*21*) of the Glu^{140}-Tyr^{141}-Val^{142} region of the S1 subunit of pertussis toxin predicted beta structure that the four–amino acid insertion of *ptx*A3201 would interrupt with a turn.

The toxin molecules produced by the codon insertion mutant strain, TOX3201, and the parental strain, BP370 (*18*), were purified from culture supernatant by fetuin-Sepharose affinity chromatography (*22*) for comparison by SDS–polyacrylamide gel electrophoresis (*23*) and Western immunoblot (*24*) (Fig. 2). The toxin molecule produced by TOX3201, which we designated CRM3201, has an S1 subunit of a larger apparent molecular weight than the native toxin S1 subunit. This appropriately reflected the insertion of four amino acids into the S1 polypeptide of CRM3201. The CRM3201 molecule was also found to contain the polypeptides of the toxin B oligomer, S2 through S5, and was found to be equivalent to the native toxin in its ability to hemagglutinate goose erythrocytes (*22*). CRM3201 had, however, only 1% of the ADP-ribosyltransferase activity of the native toxin as assayed by the ADP-ribosylation of transducin (*25*). The *ptx*A3201 mutation thus may define a region of the S1 polypeptide involved in this enzymatic activity. In sum, these data suggest that the CRM3201 toxin molecule is exported as an assembled holotoxin with a functional B oligomer but a substantially less active S1 ADP-ribosyltransferase subunit.

In assays for biological activities, several other *B. pertussis* strains were selected for comparison with TOX3201 (Fig. 1). These included a nontoxinogenic strain, TOX3311 (*18*), containing a kanamycin resistance (*kan*^{r}) gene (*26*) inserted in place of the toxin operon, and two toxinogenic *B. pertussis* strains, TOX5105 and TOX5167 (*18*), containing insertions of the *kan*^{r} gene outside of the toxin operon. We also tested strain TOX058, in which the 3′ half of the S1 gene, from the Sal I to the Xba I restriction sites, had been deleted. The construction and characterization of TOX058 will be described in detail elsewhere (*27*). The *B. pertussis* strains containing all of these mutations were derived from our virulent lab strain BP370.

The induction of leukocytosis in mice (*4*) by the *B. pertussis* strains was measured 4 days after an intravenous (IV) injection of the strains (Fig. 3A). Mice injected with strains producing the native toxin, BP370 and TOX5105, developed a dose-dependent leukocytosis. Curiously, strain TOX5105, which contains an insertion of the *kan*^{r} gene outside of the toxin structural genes, appeared slightly less potent in promoting leukocytosis. This may reflect a genetic effect of this particular insertion mutation or a physiological effect of the *kan*^{r} gene product on toxin export or assembly. In contrast, the codon insertion mutant TOX3201, as well as TOX058 and the nontoxinogenic TOX3311, induced essentially no leukocytosis.

We measured the potentiation of anaphylaxis to two different antigens, chicken egg albumin (EA) in CFW mice (*4*) and bovine serum albumin (BSA) in BALB/c mice (*14, 15*). The CFW mice were given concomitant intraperitoneal (IP) injections of EA and heat-killed *B. pertussis*, and sensitization was indicated by a lethal anaphylaxis upon IV challenge with EA 14 days later (Fig. 3B). The native toxin-producing strains BP370 and TOX5105 displayed a dose-dependent sensitizing activity. Similar to the leukocytosis induction, the *kan*^{r} insertion mutant TOX5105 was less potent. In contrast, the codon insertion mutant, TOX-3201, the S1 deletion mutant, TOX058, and the nontoxinogenic TOX3311 were all ineffective in potentiating anaphylaxis. The mice were sensitized to BSA-induced anaphylaxis by injection, for 4 days, on alternating days, with BSA and with the *B. pertussis* strains. Anaphylaxis was induced by injecting mice 5 to 7 days after the sensitization regimen with BSA. In the BSA sensitization challenge, we substituted *B. pertussis* strain TOX5167 for TOX5105. TOX5167 also contains an insertion of the *kan*^{r} gene, also

Table 1. Potentiation of BSA anaphylaxis and stimulation of histamine sensitivity. BSA anaphylaxis has been referred to as pertussis vaccine encephalopathy (*14*). *Bordetella pertussis* strains were prepared and administered as reported for histamine challenge (*4*) and BSA challenge (*14*). ND, not done.

Strains	CFU × 10^9	BSA challenge (deaths/total)*	Histamine challenge (deaths/total)*
BP370	10		10/10
	5	17/29 (59%)	ND
	2		10/10
	0.4		10/10
	0.08		3/10
TOX5167	5	26/29 (90%)	ND
TOX5105	9.4		10/10
	2		10/10
	0.4		2/10
	0.08		0/10
TOX3311	9.4		2/10
	5	0/30 (0%)	ND
	2		0/10
	0.4		0/10
	0.08		0/10
TOX3201	10		1/9
	5	0/30 (0%)	ND
	2		0/10
	0.4		0/10
	0.08		0/10
TOX058	9.2		2/9
	5	0/10 (0%)	ND
	2		0/9
	0.4		1/9
	0.08		0/9
PBS only	0.0	ND	0/19
PTX†		71/83 (86%)	ND

*Deaths/total represents the ratio of the number of animals in which the sensitization was lethal to the total number tested. †Pertussis toxin (100 ng, List Biological Laboratories) was administered in place of *B. pertussis* strain.

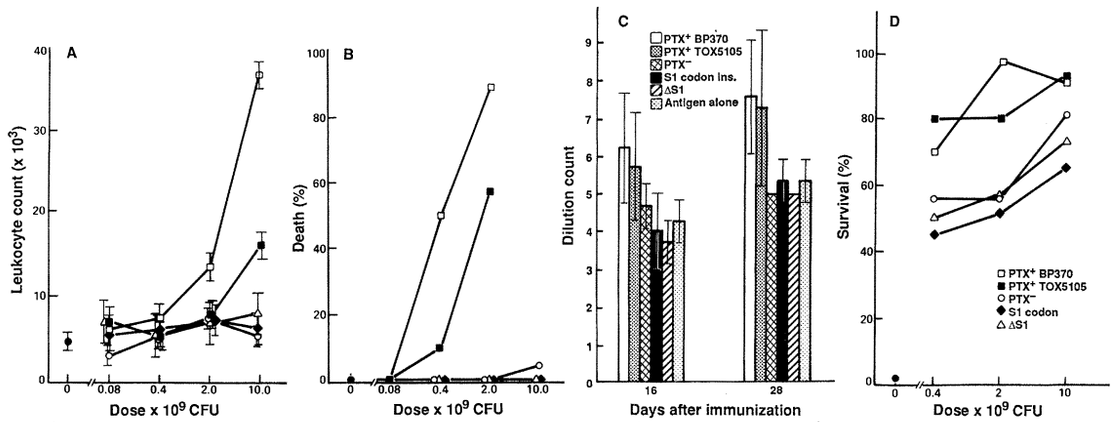

Fig. 3. Leukocytosis, anaphylaxis, adjuvanticity, and ICC. A key to the strains is presented in (D). The strains were: PTX$^+$, BP370; PTX$^+$, TOX5105; PTX$^-$, TOX3311; S1 codon, TOX3201; and ΔS1, TOX058. Controls are presented as a *B. pertussis* dose of 0 CFU. (**A**) Leukocytosis. Leukocytosis was measured (*4*) in a Coulter counter 4 days after IV injection of *B. pertussis* vaccines (*16*). Values represent leukocyte count per cubic millimeter and are averages from five animals; bars represent 1 SD. (**B**) Anaphylaxis. CFW mice were sensitized (*4*) to EA with an IP dose of 1.0 mg of the antigen and an IV dose of the *B. pertussis* strains. Mice were challenged 14 days later with 1.0 mg of EA given IV. Results are the percentage of mice that died of anaphylaxis. For each graph value the number of animals was ≥10. (**C**) Adjuvanticity. C57BL/10 SCN mice received 1.0 mg of EA IP and 2 × 10^9 CFU of heat-killed *B. pertussis* IV on day 0. On day 21, mice received a second IP injection of 5 μg of EA. Mice were bled on days 16 and 28, and we titrated sera for anti-EA by means of enzyme-linked immunosorbent assay (ELISA) using microtiter plates coated with EA. Mice receiving EA antigen without any *B. pertussis* are indicated as antigen alone. Each value represents three animals; bars represent 1 SD. (**D**) ICC. Intracerebral challenge protection. Three-week-old CFW mice immunized IP with *B. pertussis* prepared as vaccines (*16*) were challenged intracerebrally 14 days later with 10^5 CFU of *B. pertussis* strain 18323 (*16*). Values are presented as percent survival of challenged mice, and each represents at least 15 animals.

outside of the toxin operon (Fig. 1). We found that whereas a dose of 5 × 10^9 colony-forming units (CFU) of *B. pertussis* strains BP370 and TOX5167 led to a high percentage of sensitization, the *ptx* mutant strains TOX3201, TOX058, and TOX3311, were entirely unable to potentiate an anaphylactic response to BSA (Table 1).

The sensitization of mice to a lethal challenge with the vasoactive amine histamine has also been proposed to reflect a direct action of the B oligomer, in this instance, on the vascular endothelium to increase vascular permeability (*8*). The sensitizing activities of our *B. pertussis* strains were determined by injecting mice IV with heat-killed *B. pertussis* followed 4 days later by IP challenge with histamine (*4*). The toxinogenic strains BP370 and TOX5105 increased the sensitivity to histamine in a dose-dependent fashion (Table 1). The mutant strains TOX3201, TOX058, and TOX3311, in contrast, were substantially free of this activity. Thus, our data suggest that with regard to induction of leukocytosis, potentiation of anaphylaxis, and stimulation of histamine sensitivity, a *B. pertussis* strain producing an assembled holotoxin that is reduced in ADP-ribosyltransferase activity is reduced in pathogenic potential to the level of a nontoxinogenic organism.

The adjuvanticity of pertussis toxin in experimental animal models is well documented (*4, 28*) and may contribute functionally to the efficacy of the whole-cell pertussis vaccine (*1, 2*). The role of the ADP-ribosyltransferase activity in the adjuvant action, however, has been disputed (*8*); we therefore tested the mutants for their adjuvant activity in the production of antibodies to the antigen EA (Fig. 3C). C57BL/10 SCN mice were injected concomitantly with EA and heat-killed *B. pertussis* and were measured 14 days later for anti-EA titers. The toxinogenic parental strain, BP370, exhibited a marked adjuvant action on the production of antibody to EA. The titers were increased further by a small secondary injection of EA given on day 21. The toxinogenic *kan*^{r} strain, TOX5105, also manifested an adjuvant action, though it was less apparent until after the secondary immunization of EA. In contrast, concomitant injection of EA with the *ptx* mutant strains TOX3201, TOX058, or TOX3311 showed no adjuvant effect after either the primary or secondary injection.

To further investigate the loss of immunostimulation seen with the *ptx* mutants, their ability to protect mice from a lethal intracerebral challenge (ICC) infection with *B. pertussis* (*16*) was studied. Though it was apparent that the mutations in the S1 subunit gene would interfere functionally with the adjuvant activity of *B. pertussis*, we felt that the assembled and exported CRM3201 holotoxin molecule of TOX3201 might still serve, at least structurally, as an efficacious immunogen. The ICC infection is used to assay the potency of pertussis vaccine preparations in the United States, and involves IP immunization of test mice with whole-cell vaccine preparations, followed 2 weeks later by an ICC with the standard virulent strain of *B. pertussis*, 18323 (*16*). Both the wild-type and the mutant strains of *B. pertussis* provided a dose-dependent degree of protection against ICC infection (Fig. 3D). However, the dose-response curves for the *ptx* mutant strains TOX3201, TOX058, and TOX3311 were lower than those of the strains producing the native toxin. At the highest immunizing dose, protection with the native toxin-producing strains approached 100% of a cohort, whereas at similar doses the S1 mutants and the nontoxinogenic mutant induced only about 70% protection. This would seem to indicate that the ADP-ribosyltransferase activity is critical for optimum immunoprotection. An alternative explanation, that the region of the S1 polypeptide that we altered with *ptx*A3201 may be a critical structural epitope, is unlikely since it has been shown that an S1 polypeptide alone containing the native Glu^{140}-Tyr^{141}-Val^{142} region is an inefficacious immunogen (*29*).

Taken together, our results regarding leukocytosis, anaphylaxis, adjuvanticity, and immunoprotection of mice from an ICC infection suggest that the ADP-ribosyltransferase activity of pertussis toxin correlates directly with the immunomodulatory activities of a *B. pertussis* strain. TOX3201 produces an assembled holotoxin with a reduced ADP-ribosyltransferase activity and was reduced in these immunopathologic and immunoprotective activities. These data imply that mutations in the toxin genes that reduce pathogenic activities of a strain such as leukocytosis can also reduce the immunoprotective capacity of the strain. This is an important consideration in the formulation of future pertussis vaccines.

REFERENCES AND NOTES

1. A. C. Wardlaw and R. Parton, *Pharmacol. Ther.* **19**, 1 (1983).
2. M. Pittman, *Pediatr. Infect. Dis.* **3**, 467 (1984).
3. A. A. Weiss and E. L. Hewlett, *Annu. Rev. Microbiol.* **40**, 661 (1986).
4. J. J. Munoz, H. Arai, R. K. Bergman, P. L. Sadowski, *Infect. Immun.* **33**, 820 (1981).
5. M. Tamura *et al.*, *Biochemistry* **21**, 5516 (1982).

6. G. M. Bokoch, T. Katada, J. K. Northup, E. L. Hewlett, A. G. Gilman, *J. Biol. Chem.* **258**, 2072 (1983); T. Katada, M. Tamura, M. Ui, *Arch. Biochem. Biophys.* **224**, 290 (1983); T. Murayama and M. Ui, *J. Biol. Chem.* **258**, 3319 (1983).
7. M. Tamura *et al.*, *J. Biol. Chem.* **258**, 6756 (1983).
8. M. Ui, K. Nogimori, M. Tamura, in *Pertussis Toxin*, R. D. Sekura, J. Moss, M. Vaughan, Eds. (Academic Press, Orlando, FL, 1985), pp. 19–43; K. Nogimuri *et al.*, *Dev. Biol. Stand.* **61**, 51 (1985).
9. M. Granstrom, G. Granstrom, P. Gillenius, P. Askelof, *J. Infect. Disease* **151**, 646 (1985).
10. Y. Sato, H. Kimura, H. Fukumi, *Lancet* **1984-I**, 122 (1984); G. R. Noble *et al.*, *J. Am. Med. Assoc.* **257**, 1351 (1987).
11. J. D. Cherry, *Curr. Clin. Top. Infect. Dis.* **7**, 216 (1986).
12. C. Locht and J. M. Keith, *Science* **232**, 1258 (1986); A. Nicosia *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4631 (1986).
13. S. I. Morse and J. H. Morse, *J. Exp. Med* **143**, 1483 (1971).
14. L. Steinman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 8733 (1985).
15. J. J. Munoz, M. G. Peacock, W. J. Hadlow, *Infect. Immun.* **55**, 1004 (1987); K. Redhead, A. Robinson, L. A. E. Ashworth, M. Melville-Smith, *J. Biol. Stand.*, in press.
16. P. Kendrick, G. Eldering, M. K. Dixon, J. Misner, *Am. J. Public Health* **37**, 803 (1947); P. Kendrick, E. L. Updyke, G. Eldering, *ibid.* **39**, 179 (1949).
17. F. Heffron, M. So, B. J. McCarthy, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 6012 (1978).
18. W. J. Black and S. Falkow, *Infect. Immun.* **55**, 2465 (1987).
19. S. Stibitz, W. J. Black, S. Falkow, *Gene* **50**, 133 (1986).
20. S. F. Carroll *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7237 (1985); S. F. Carroll and R. J. Collier, *J. Biol. Chem.* **262**, 8707 (1987).
21. P. Y. Chou and G. D. Fasman, *Adv. Enzymol. Relat. Areas Mol. Biol.* **47**, 45 (1978).
22. Y. Sato, J. L. Cowell, H. Sato, D. G. Burstyn, C. R. Manclark, *Infect. Immun.* **41**, 313 (1983); R. D. Sekura, F. Fish, C. R. Manclark, B. Meade, Y. Zhang, *J. Biol. Chem.* **258**, 14647 (1983).
23. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
24. W. N. Burnette, *Anal. Biochem.* **112**, 195 (1981).
25. P. A. Watkins *et al.*, *J. Biol. Chem.* **260**, 13478 (1985).
26. A. Oka, H. Sugasaki, M. Takanami, *J. Mol. Biol.* **147**, 217 (1981).
27. W. J. Black *et al.*, in preparation.
28. A. Robinson and L. I. Irons, *Infect. Immun.* **40**, 523 (1983).
29. A. Nicosia, A. Bartoloni, M. Perugini, R. Rappuoli, *ibid.* **55**, 963 (1987).
30. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
31. This work was supported by a Bank of America Giannini Foundation Fellowship to W.J.B., NIH grant AI-23945 to S.F., and NIH grant AI-22462 to L.S.

6 October 1987; accepted 26 February 1988

Immunotherapy of the Nonobese Diabetic Mouse: Treatment with an Antibody to T-Helper Lymphocytes

JUDITH A. SHIZURU, CARIEL TAYLOR-EDWARDS, BETH ANN BANKS, ANITA K. GREGORY, C. GARRISON FATHMAN

Spontaneous diabetes mellitus was blocked in nonobese diabetic mice by treatment with a monoclonal antibody against the L3T4 determinant present on the surface of T-helper lymphocytes. Sustained treatment with the monoclonal antibody led to cessation of the lymphocytic infiltration associated with the destruction of the insulin-producing β cells. Moreover, the mice remained normoglycemic after the antibody therapy was stopped. These studies indicate that immunotherapy with monoclonal antibodies to the lymphocyte subset may not only halt the progression of diabetes, but may lead to long-term reversal of the disease after therapy has ended.

THERE IS INCREASING EVIDENCE that human insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease and that IDDM results from immune destruction of the insulin-producing β cells normally found in the islets of Langerhans (*1*). Nonobese diabetic (NOD) mice spontaneously develop diabetes (*2*) resembling human IDDM. As in human IDDM, the NOD mice have progressive lymphocytic infiltration into the islets (insulitis) before the expression of overt diabetes (*2–4*), and cytoplasmic antibodies to islet cells appear in their serum during the development of insulitis (*4, 5*). Susceptibility to diabetes in both humans and NOD mice is strongly associated with genes of the major histocompatibility complex (MHC) (*6*). Overt diabetes is characterized by polyuria, polydipsia, hyperglycemia, and glycosuria, and NOD mice develop acute ketoacidosis, which is fatal unless the mice are treated with insulin (*2, 7*).

The specific immunologic pathways and cell types responsible for islet cell destruction in NOD mice are not clearly delineated (*8*). However, recent studies suggest that the T lymphocyte subset that expresses the L3T4 surface marker is important in the pathogenesis of the disease (*9*). T lymphocytes of the L3T4 phenotype are a distinct subpopulation of mature T cells that function as helper-inducer cells in the activation of both humoral and cellular immunity (*10*). The L3T4 lymphocyte subset is responsible for MHC class II–restricted antigen recognition on antigen-presenting cells (*11*); the human homolog to the murine $L3T4^+$ T cell is the $CD4^+$ T cell (*11*). We have been able to block the progression and subsequent expression of overt diabetes in NOD mice by a course of treatment with a monoclonal antibody to L3T4. Such an approach may be feasible for treatment of patients with subclinical manifestations of IDDM, since we show that antibody therapy initiated late in disease progression was effective in reversing the advanced phases of islet cell destruction. Moreover, upon cessation of therapy the mice have remained disease-free without further treatment.

Department of Medicine, Division of Immunology, Stanford University Medical Center, Stanford, CA 94305–5111.

The monoclonal antibody used in these studies, GK1.5, is a cell-depleting antibody. When administered to mice at doses greater than 300 μg, this antibody causes sustained reduction of more than 90% of the circulating $L3T4^+$ cells (*12*). GK1.5 has been successfully used in vivo as an immunotherapeutic agent to treat other experimental and spontaneous autoimmune diseases, including systemic lupus erythematosus (*13*), experimental allergic encephalomyelitis (*14*), and type II collagen-induced arthritis (*15*). In addition, a single course of this antibody has been shown to allow indefinite acceptance of transplanted allogeneic murine islets of Langerhans (*16*). GK1.5 and other antibodies to L3T4 are particularly suitable for serotherapy, since these reagents can suppress the humoral immune response (*12, 17*) and induce tolerance to select protein antigens, including the monoclonal antibody to L3T4 itself (*17*).

When NOD mice are 30 to 50 days old, mononuclear cells begin to infiltrate the perivascular and periductal areas around the

Table 1. Prevention of diabetes in NOD mice by long-term treatment with GK1.5. Rat monoclonal antibody GK1.5 (immunoglobulin G2b) to mouse L3T4, purified from ascites fluid, was administered intraperitoneally to 90- to 110-day-old NOD female mice. Incidence of diabetes is shown as the ratio of the number of diabetic mice to total number of mice in the group at 260 days of age.

Amount of GK1.5 administered (μg)	Incidence of diabetes	Time of onset of diabetes (days)
600	18/21	157 ± 33
600, then 100 weekly	2/25	156 ± 43
None	29/35	173 ± 42