that the prevention of diabetes by intrathymic islet implantation was not due to such nonspecific alterations in T cell immunity by performing several in vivo and in vitro assays on recipients of intrathymic islets. Total cell numbers as well as phenotypic distributions of T cell subsets in the peripheral lymph nodes of islet-grafted animals were not significantly different from those of saline-treated and unmanipulated BB rats, and all groups exhibited the T lymphopenia characteristic of this strain (Table 2). Similarly, mixed lymphocyte culture and concanavalin A (Con A)–stimulated proliferative responses of lymph node cells from experimental and saline-treated BB rats were comparable, all being reduced as compared with responses of lymphoid cells that were not from the BB strain (*17, 18*). In vivo T cell–mediated responses were also present to a similar degree in experimental and control rats, as confirmed by the capacity of islet-grafted animals to reject allogeneic Lewis ($RT1^l$) skin grafts at a rate comparable to that observed in control diabetics (13 and 35 days versus 14, 22, 29, and 34 days, respectively).

These results indicate that the prevention of autoimmune diabetes by neonatal islet transplantation is β cell–specific, is not dependent on systemic alterations in immune function that have been associated with other methods of preventing diabetes in the BB rat, and requires that the islet implant be situated in the thymic microenvironment.

Prophylactic administration of high doses of insulin to normoglycemic, prediabetic BB rats reduces their incidence of diabetes, possibly by rendering the endogenous islets metabolically inactive and thus decreasing their expression of β cell–specific autoantigens (*19–21*). This mechanism seems an unlikely explanation for the prevention of insulitis and diabetes that we observed because of the small number of islets implanted in the thymus and the inability of renal subcapsular islet grafts to prevent disease. Furthermore, in the reports mentioned above, insulin therapy provided only partial protection from diabetes and did not prevent development of insulitis, whereas we observed complete prevention of both hyperglycemia and insulitis after neonatal intrathymic islet transplantation.

A more likely explanation for our findings is that intrathymic transplantation of islets into neonatal BB rats alters T cell development by promoting the deletion or functional inactivation of antigen-specific clones before their migration to the periphery (*22–24*). Such an effect could be mediated by interactions of maturing host T cells with islets expressing the β cell autoantigen–MHC complex or with host-derived thymic antigen presenting cells bearing processed β cell antigens that were shed from the islet graft. Alternatively, the long-term residence of islets in the thymus may stimulate the selection of specific regulatory cells capable of suppressing anti-islet autoimmune responses.

Our data show that introduction of a small number of MHC-compatible islets into the thymus of prediabetic BB rats prevents the development of spontaneous diabetes, an effect that appears to result from specific regulation of anti-islet autoimmunity. These findings could lead to the development of novel approaches for the prevention of diabetes and other organ-specific autoimmune diseases.

REFERENCES AND NOTES

1. A. M. Posselt *et al.*, *Science* **249**, 1293 (1990).
2. A. M. Posselt, A. Naji, J. H. Roark, J. F. Markmann, C. F. Barker, *Ann. Surg.* **214** (no. 4), 363 (1991).
3. A. F. Nakhooda, A. A. Like, G. I. Chappel, F. T. Murray, E. B. Marliss, *Diabetes* **26**, 100 (1977).
4. N. A. Parfrey *et al.*, *Crit. Rev. Immunol.* **9** (no. 1), 45 (1989).
5. B. M. Dean, R. Walker, A. J. Boen, J. D. Baird, A. Cook, *Diabetologia* **28**, 464 (1985).
6. U. McKeever *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7618 (1990).
7. J. P. Mordes, J. Desemone, A. A. Rossini, *Diabetes Metab. Rev.* **3** (no. 3), 725 (1987).
8. In our colony of BB rats, the cumulative incidence of diabetes was 40 to 60%, with most (>99%) of the animals developing the disease between 50 and 165 days of age.
9. A. A. Like *et al.*, *J. Exp. Med.* **164**, 1145 (1986).
10. J. A. Jaworski *et al.*, *Diabetes Res.* **3**, 1 (1986).
11. A. A. Rossini *et al.*, *J. Clin. Invest.* **74**, 36 (1984).
12. A. A. Like, A. A. Rossini, D. L. Guberski, M. C. Appel, *Science* **206**, 1421 (1979).
13. N. Murase *et al.*, *Diabetes* **39**, 1584 (1990).
14. A. Naji, W. K. Silvers, D. Bellgrau, C. F. Barker, *Science* **213**, 1390 (1981).
15. J. P. Mordes *et al.*, *Diabetologia* **30**, 22 (1987).
16. A. Naji *et al.*, *J. Immunol.* **130**, 2168 (1983).
17. Proliferative responses (counts per minute ± SE) of lymph node cells (3×10^5 per well) from WF rats, BB recipients of intrathymic WF islets, and control diabetic BB littermates that were cultured with irradiated Lewis ($RT1^l$) stimulators (3×10^5 per well) were: 66,354 ± 5,245, 6,279 ± 887, and 7,595 ± 92, respectively (n = 3 to n = 4 per group). Responses of cells cultured without stimulators were: 11,865 ± 1,855, 6,350 ± 542, and 5,184 ± 1,489, respectively. Responses of WF, islet-grafted BB, and control diabetic BB rat splenocytes (5×10^5 per well) cultured alone or in the presence of Con A (5 μg/ml) for 72 hours were: 8,879 ± 2,177 and 424,747 ± 63,891, 5,811 ± 2,061 and 3,523 ± 1,355, and 7,149 ± 768 and 5,355 ± 1,207, respectively (n = 3 per group).
18. H. Georgiou, A. C. Lagarde, D. Bellgrau, *J. Exp. Med.* **167**, 132 (1988).
19. C. F. Gotfredsen, K. Buschard, E. K. Frandsen, *Diabetologia* **28**, 933 (1985).
20. A. A. Like, *Diabetes* **35** (suppl. 1), 74A (1986).
21. M. C. Appel, F. Dotta, J. J. O'Neill, G. S. Eisenbarth, *Diabetologia* **32**, 461A (1989).
22. P. Kisielow, H. Blüthmann, U. D. Staerz, M. Steinmetz, H. von Boehmer, *Nature* **333**, 742 (1988).
23. F. Ramsdell, T. Lantz, B. J. Fowlkes, *Science* **246**, 1038 (1989).
24. R. H. Schwartz, *Cell* **57**, 1073 (1989).
25. T. Hunig, H.-J. Wallny, J. K. Hartley, A. Lawetzky, G. J. Tiefenthaler, *J. Exp. Med.* **169**, 73 (1989).
26. D. W. Mason *et al.*, *Immunol. Rev.* **74**, 57 (1983).

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Conjugative Transfer by the Virulence System of *Agrobacterium tumefaciens*

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Agrobacterium tumefaciens transfers part of its Ti plasmid, the transferred DNA (T-DNA), to plant cells during tumor induction. Expression of this T-DNA in plant cells results in their transformation into tumor cells. There are similarities between the process of T-DNA transfer to plants and the process of bacterial conjugation. Here, the T-DNA transfer machinery mediated conjugation between bacteria. Thus, products of the Vir region of the Ti plasmid of *Agrobacterium tumefaciens*, normally involved in transfer of DNA from bacteria to plants, can direct the conjugative transfer of an IncQ plasmid between agrobacteria.

The genes responsible for T-DNA transfer from *Agrobacterium tumefaciens* to plant cells are located in the Vir region of the Ti plasmid and in the bacterial chromosome (*1*). There are many similarities between T-DNA transfer and bacterial conjugation, including the introduction of single-stranded breaks in the DNA molecules that are transferred. In T-DNA transfer, the proteins VirD1 and VirD2, encoded by the *virD* operon of the Vir region, together act as an endonuclease on the bottom strands of the border sequences that surround the T region (*1*). In mobilizable plasmids, such as RSF1010, nicks are produced by Mob proteins at the origin of transfer (*oriT*), and there is sequence homology between border repeats and the *oriT* sequences of certain plasmids (*2*). Conjugative transfer involves

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transport of single-stranded DNA. Likewise, T-DNA processing results in the formation of single-stranded DNA molecules (*1*). Plasmid RSF1010, which lacks a T region border repeat, carries an *oriT* sequence and encodes Mob proteins necessary for transfer into plant cells from *Agrobacterium* strain LBA4404 (*3*). This suggests that nicking of *oriT* by Mob proteins can replace border repeat nicking by the VirD proteins in the T-DNA transfer process.

In bacterial conjugation, transport of single-stranded plasmid DNA from donor to recipient probably occurs through membrane pores and involves pili (*4*), both encoded by the transfer (*tra*) genes present on conjugative plasmids. It has been hypothesized that during T-DNA transfer DNA may also be transported across membranes through a porelike structure. Proteins encoded by the *virB* operon, a *vir*-operon essential for T-DNA transfer to

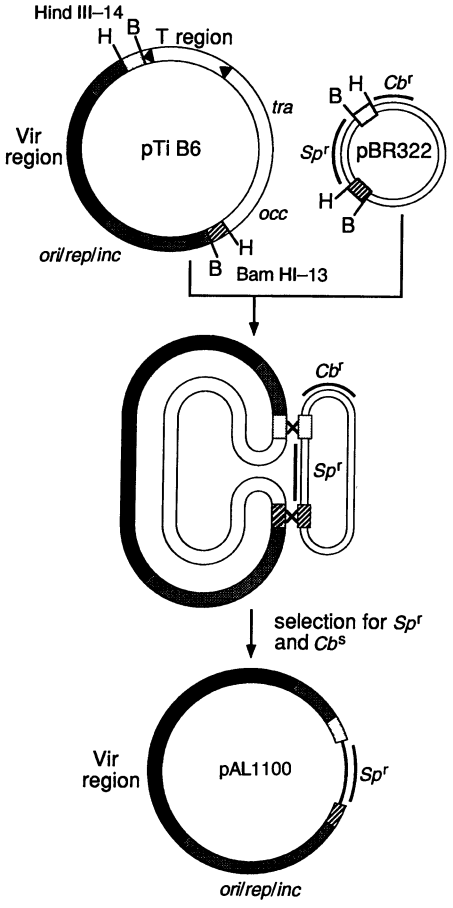

Fig. 1. Strategy for the construction of the helper plasmid pAL1100. A Hind III–Bam HI fragment (2.5 kb) from Hind III–14, a 2.4-kb fragment (*Sp*^{r} gene from Tn*1831*), and a Hind III–Bam HI fragment (4.2 kb) from Bam HI–13 were cloned into pBR322. After introducing this plasmid into *A. tumefaciens* LBA1010, we selected colonies for resistance to rifampicin (*Rif*^{r}) and to spectinomycin (*Sp*^{r}). To detect strains in which a double crossover had occurred, we screened the colonies for sensitivity to carbenicillin (*Cb*^{s}). Plasmid DNA was isolated (*26*) from putative recombinants, digested with restriction enzymes, and analyzed by Southern (DNA) blot (*25*); *tra*, transfer region; *occ*, octopine catabolism; *ori*, origin of replication; *rep*, replication region; *inc*, incompatibility region; H, Hind III site; B, Bam HI site.

Table 1. *Agrobacterium* strains used. Δ indicates deletion. T_L, left T region; T_R, right T region.

Strain	Chromosomal background	Resistance markers	Plasmid
LBA288 (*29*)	C58	Rif	Without Ti
LBA1010 (*30*)	C58	Rif	Wild-type pTiB6
LBA1100	C58	Rif, Sp	pAL1100 or pTiB6 ΔT_L, ΔT_R, Δ*tra*, Δ*occ*
LBA1141	C58	Rif, Sp, Cb	pAL1100 (*virH*::Tn*3Hoho1*)
LBA1142	C58	Rif, Sp, Cb	pAL1100 (*virA*::Tn*3Hoho1*)
LBA1143	C58	Rif, Sp, Cb	pAL1100 (*virB4*::Tn*3Hoho1*)
LBA1144	C58	Rif, Sp, Cb	pAL1100 (*virB7*::Tn*3Hoho1*)
LBA1145	C58	Rif, Sp, Cb	pAL1100 (*virG*::Tn*3Hoho1*)
LBA1146	C58	Rif, Sp, Cb	pAL1100(*virC2*::Tn*3Hoho1*)
LBA1148	C58	Rif, Sp, Cb	pAL1100 (*virD4*::Tn*3Hoho1*)
LBA1149	C58	Rif, Sp, Cb	pAL1100 (*virE2*::Tn*3Hoho1*)
LBA2329 (*31*)	C58	Ery	Without Ti
LBA2885 (*30*)	C58	Rif, Cb	pAL2885 or pTiB6 Δ*vir*, ΔT_L, ΔT_R, Δ*tra*, Δ*occ*
LBA4011 (*32*)	Ach5	Rif	Without Ti
LBA4020 (*32*)	Ach5	Sp, Ery	Without Ti
LBA4404 (*12*)	Ach5	Rif	pAL4404 or pTiAch5 ΔT_L, ΔT_R, Δ*tra*, Δ*occ*

Table 2. Conjugation experiments with strains that contained Ti plasmid derivatives or *vir*-mutated Ti helper plasmids as donors. For LBA4404 and LBA4011 the acceptor strain was LBA4020 and for LBA2885 and LBA1100 the acceptor strain was LBA2329. In all cases the recipient strain was LBA2329, and no transfer was detected in IM without an inducer. All donor strains contained pKT230; IM, induction medium; IM + AS, induction medium with AS (*13*). The titer of input donors and recipients was determined immediately after mixing; the titer of recovered donors and recipients from the suspension in PZ (110 μl) was determined after 3 days of incubation (*13*). Data represent similar data obtained in at least a dozen independent experiments.

Donor strain	Medium	Titer input (× 10^7 cells/ml)		Titer recovered (× 10^7 cells/ml)		Recovered trans-conjugants in 100 μl (*n*)	Conjugation frequency per input donor
		Donor	Recipient	Donor	Recipient		
Experiment with strains with Ti plasmid derivatives							
LBA4404	IM	1.2	2.5	48.2	55.9	0	$<1.7 \times 10^{-6}$
	IM + AS	0.7	2.5	4.7	86.7	7	2.2×10^{-5}
LBA4011	IM	2.3	2.5	82.0	33.9	0	$<8.6 \times 10^{-7}$
	IM + AS	2.2	2.5	110.6	36.0	0	$<9.2 \times 10^{-7}$
LBA2885	IM	3.5	2.4	16.0	17.8	0	$<5.7 \times 10^{-7}$
	IM + AS	3.8	2.4	17.1	3.6	0	$<5.5 \times 10^{-7}$
LBA1100	IM	3.6	2.4	17.6	14.9	0	$<5.5 \times 10^{-7}$
	IM + AS	2.2	2.4	6.9	3.8	95	9.5×10^{-5}
Experiment with strains with vir*-mutated Ti helper plasmids*							
LBA1100	IM	2.3	2.4	6.0	11.4	0	$<4.4 \times 10^{-7}$
	IM+ AS	1.9	2.6	5.9	9.6	321	1.9×10^{-4}
LBA1141	IM	1.8	2.6	7.1	17.2	0	$<5.4 \times 10^{-7}$
	IM + As	1.5	2.5	3.9	10.7	98	7.0×10^{-5}
LBA1142	IM	2.0	2.6	13.1	16.1	0	$<5.0 \times 10^{-7}$
	IM + AS	2.0	2.6	17.6	8.4	0	$<4.9 \times 10^{-7}$
LBA1143	IM	2.5	2.6	10.4	16.6	0	$<4.0 \times 10^{-7}$
	IM + AS	1.5	2.6	4.8	8.4	0	$<6.7 \times 10^{-7}$
LBA1144	IM	1.9	2.3	10.9	21.8	0	$<5.2 \times 10^{-7}$
	IM + AS	1.3	2.3	6.4	9.7	0	$<7.5 \times 10^{-7}$
LBA1145	IM	1.6	2.4	11.3	16.3	0	$<6.4 \times 10^{-7}$
	IM + AS	2.3	2.2	5.6	5.0	0	$<4.4 \times 10^{-7}$
LBA1146	IM	1.0	2.2	3.9	19.7	0	$<9.8 \times 10^{-7}$
	IM + AS	1.6	2.3	6.3	14.1	137	9.6×10^{-5}
LBA1148	IM	1.2	2.2	0.8	28.3	0	$<8.1 \times 10^{-7}$
	IM + AS	0.8	2.1	0.1	14.4	0	$<1.2 \times 10^{-6}$
LBA1149	IM	1.1	2.0	10.4	15.6	0	$<8.8 \times 10^{-7}$
	IM + AS	1.5	2.1	5.4	11.3	218	1.6×10^{-4}

plants, are thought to be localized in the membrane (5–9). The VirB11 protein has adenosine triphosphatase activity and is homologous to the first open reading frame (ORF) of the *Bacillus subtilis comG* operon, which is responsible for uptake of single-stranded DNA (*9*, *10*). Although the function of the VirB proteins is unknown, their predicted membrane topologies suggest a possible function in the formation of a membrane pore through which the T-DNA (probably complexed with proteins) is transported. Here we analyze the ability of the Vir proteins to substitute for Tra proteins in the conjugal transfer of an IncQ plasmid between agrobacteria.

The wide host range IncQ plasmid pKT230 is a derivative of RSF1010 and, like its parent, is nonconjugative but can be mobilized by certain conjugative plasmids (*11*). It encodes mobilization proteins that are active at its own *oriT* but needs a conjugative plasmid such as RP4 (IncP) for other transfer functions. As expected, in our experiments LBA4404, which contains the transfer-deficient Ti plasmid pAL4404 (*12*), did not mobilize pKT230 during incubations on standard poor or rich media (Tables 1 and 2) (*13*).

However, when this strain was incubated on minimal medium that contained the *vir*-inducer acetosyringone (AS), mobilization of the IncQ plasmid did occur at a frequency of 2×10^{-5} per input donor (Table 2). None of the colonies found on medium selective for transconjugants was rifampicin-resistant, which shows that these colonies represented true transconjugants and not donors that had become spectinomycin-resistant spontaneously. The presence of the deleted Ti plasmid was essential for mobilization because strain LBA4011, which is identical to LBA4404 but lacks the deleted Ti plasmid, could not transfer pKT230 (Tables 1 and 2). It is possible that transfer occurs after secretion of plasmid DNA into the medium and uptake by the recipient. However, the addition of deoxyribonuclease (DNase; 1 μg/ml; Sigma) to the medium did not lower the transfer frequency, which indicates that transfer does not occur by way of transformation. Strain LBA2885, in which the area missing in pAL4404 and also the Vir region of the Ti plasmid are deleted, did not mobilize pKT230 to recipients (Tables 1 and 2). These experiments together indicate that the Vir system can mediate conjugation between agrobacteria.

To analyze involvement of the different *vir* loci, we constructed a transfer-deficient (*tra*$^-$) helper plasmid that determines spectinomycin resistance (*Sp*^{r}) in a C58 background (Fig. 1) and introduced mutations in the Vir region of this helper plasmid (Table 1) (*14*, *15*). Strain LBA1100, which contains this new helper plasmid, showed mobilization of pKT230 under similar conditions as LBA4404 (Table 2). Again, the colonies obtained were all rifampicin-resistant, which shows that they represented true transconjugants and not donors that had acquired erythromycin resistance spontaneously. Despite the Tra$^-$ phenotype of the strain with helper plasmid pAL1100, it is probable that it still contains some *tra* genes. This small remaining segment of the Tra region was not sufficient, however, for mobilization of the IncQ plasmid because no IncQ transfer was observed in the absence of the *vir*-inducer AS, not even during incubations on media that contained the Ti plasmid *tra*-inducer octopine.

Mutants LBA1141 and LBA1146 mobilized the IncQ plasmid on media with AS, which shows that the host range loci *virH* (*1*) and *virC* (*1*) are not required for the mobilization of pKT230. However, mutations in the operons *virA* and *virG* (Tables 1 and 2, LBA1142 and LBA1145) abolished the conjugative transfer of the IncQ plasmid. This was expected, because the regulatory proteins encoded by the *virA* and *virG* genes mediate the induction of the Vir regulon in the presence of AS (*1*). The VirE2 protein is a single-stranded DNA binding protein, which may protect the T-DNA during transfer to the plant cell nucleus (*16*). The *virE* mutant LBA1149 mobilized the IncQ plasmid normally; we therefore conclude that a single-stranded DNA binding protein similar to VirE2 is provided by the donor, recipient, or the IncQ plasmid itself or that such a protein is not necessary for the conjugative transfer of the IncQ plasmid between bacteria. Strains LBA1143 and LBA1144 (Table 1) with mutations in the *virB* operon did not mediate transfer of the IncQ plasmid to the recipient strain (Table 2).

The finding that *virB* is necessary for the conjugative transfer of pKT230 between agrobacteria suggests that VirB proteins function in the T-DNA transfer process like certain Tra proteins function in conjugation. Plasmid RSF1010 inhibits tumor formation but this inhibition can be overcome by overexpression of *virB9*, *virB10*, and *virB11* genes (*17*); in transfer to plant cells the VirB transfer machinery does not distinguish the T-DNA and RSF1010 transfer intermediates, which is in agreement with our hypothesis. Mutant LBA1148 was not able to mobilize pKT230 (Tables 1 and 2). This strain has a mutation in the most distal ORF of the *virD* operon. Thus, at least the protein VirD4 is necessary for mobilization of pKT230. This protein, located in the inner membrane of the bacterium (*18*), is essential for T-DNA transfer to plants and may be part of the complex transporting DNA across the bacterial membrane.

Previously, *vir* mutations were found to affect transfer of Ti plasmids between agrobacteria (*19*, *20*). However, these experiments were done under conditions in which the Vir system was not induced by AS. In our experiments we did not observe IncQ mobilization under conditions in which the *vir* genes had not been activated by AS; insertional mutations in specific *vir* genes completely abolished this transfer.

Our results show that the VirB and VirD4 proteins encoded by the Ti plasmid Vir region, which are normally involved in transfer of DNA from bacteria to plants, can direct the conjugative transfer of an IncQ plasmid between agrobacteria. In fact, there is homology between the VirD4 protein and the TraG protein encoded by the conjugative plasmid RP4 (*21*). Thus, the trans-kingdom transfer of DNA from *Agrobacterium* to plants may occur like the process of bacterial conjugation.

REFERENCES AND NOTES

1. P. Zambryski, J. Tempe, J. Schell, *Cell* **56**, 193 (1989); L. S. Melchers and P. J. J. Hooykaas, *Oxf. Surv. Plant Mol. Cell Biol.* **4**, 167 (1987).
2. W. Pansegrau and E. Lanka, *Nucleic Acids Res.* **19**, 3455 (1991).
3. V. Buchanan-Wollaston, J. E. Passiatore, F. Cannon, *Nature* **328**, 172 (1987).
4. N. Willetts and R. Skurray, in Escherichia coli *and* Salmonella typhimurium: *Cellular and Molecular Biology*, F. C. Neidhardt *et al.*, Eds. (American Society for Microbiology, Washington, DC, 1987), chap. 64.
5. D. V. Thompson *et al.*, *Nucleic Acids Res.* **16**, 4621 (1988).
6. J. E. Ward *et al.*, *J. Biol. Chem.* **263**, 5804 (1988).
7. P. Engström, P. Zambryski, M. Van Montagu, S. Stachel, *J. Mol. Biol.* **197**, 635 (1987).
8. J. E. Ward, E. M. Dale, E. W. Nester, A. N. Binns, *J. Bacteriol.* **172**, 5200 (1990).
9. P. J. Christie *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 9677 (1989).
10. M. Albano, R. Breitling, D. A. Dubnau, *J. Bacteriol.* **171**, 5386 (1989).
11. M. Bagdasarian *et al.*, *Gene* **16**, 237 (1981).
12. A. Hoekema, P. R. Hirsch, P. J. J. Hooykaas, R. A. Schilperoort, *Nature* **303**, 179 (1983).
13. Bacteria were grown for 16 hours at 29°C in minimal medium (MM) (*22*) with antibiotics, collected by centrifugation, and then resuspended in minimal induction medium (IM, composed of MM salts, 3% sucrose, and 62.5 mM potassium phosphate to adjust the pH to 5.3) without antibiotics and with or without 200 μM AS (in dimethyl sulfoxide) (Aldrich-Chemie, Steinheim, Germany) at an optical density (OD) at 660 nm of 0.10. The cultures were grown at 29°C for 6 hours (to $OD_{660} \approx 0.25$), then assayed for β-galactosidase activity (*23*). The induction of the virulence genes in these conditions was determined with strain LBA2525 (*24*) (*virB2::lacZ*). On average, 3500 units of β-galactosidase were obtained per milliliter of LBA2525 culture. Donor and recipient bacteria were mixed in equal numbers, and 2.5×10^6 total bacteria were collected on a filter (cellulose nitrate; 0.45 μm; Sartorius), placed on IM agar with or without 200 μM AS, and incubated for 3 days at 29°C in the presence of DNase I (1 μg/ml) (Sigma). Bacteria were then suspended in 2 ml 0.9% NaCl (w/v) (PZ), collected by centrifugation (10 min, 4000 rpm), rinsed in PZ, and plated on LB agar (*25*) that contained the appropriate antibiotics. Colonies were selected for a marker of the recipient strain (*Sp*^{r} for LBA4020) and for the transferred plasmid pKT230 [kanamycin-re-

sistant (Km^r)], or for the acceptor or recipient strain only. After 3 days of incubation at 29°C, the transfer frequencies (number of transconjugants versus the number of input donor cells) were determined, and the transconjugants were tested for rifampicin sensitivity (Rif^s) and for the presence of plasmid pKT230 by DNA analysis (*26*). Antibiotic concentrations were as follows: rifampicin (Rif) (20 μg/ml), kanamycin (Km) (100 μg/ml), carbenicillin (Cb) (75 μg/ml), spectinomycin (Sp) (250 μg/ml), erythromycin (Ery) (100 μg/ml), and gentamicin (Gm) (40 μg/ml).
14. The *virB* mutants were constructed as follows: first, plasmid pSM30 (or pSM1) (*15*), which contains a Tn*3Hoho1* insertion in the 5′ end (or 3′ end) of the *virB* operon, was introduced into LBA1100. Then, the incompatible plasmid pPH1JI was transferred to LBA1100 (pSM30 or pSM1) with selection for Cb^r (Tn*3Hoho1*) (*15*) and Gm^r (pPH1JI) (*27*). Colonies were screened for Km^s, which is indicative of a double crossover. After electroporation of the Ti plasmid DNA (*28*) to a new host (LBA288) to remove pPH1JI, plasmid pKT230 was introduced by mobilization from an *Escherichia coli* strain with pRK2013. The other *vir* helper Ti plasmids were constructed similarly, with pSM219, pSM202, pSM321, pSM364, pSM370, and pSM358 (*15*) for the construction of LBA1141, LBA1142, LBA1145, LBA1146, LBA1148, and LBA1149, respectively.
15. S. E. Stachel and E. W. Nester, *EMBO J.* **5**, 1445 (1986).
16. V. Citovsky, M. L. Wong, P. Zambryski, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 1193 (1989).
17. J. E. Ward, E. M. Dale, A. N. Binns, *ibid.* **88**, 9350 (1991).
18. S. Okamoto, A. Toyoda-Yamamoto, K. Ito, I. Tabeke, Y. Machida, *Mol. Gen. Genet.* **228**, 24 (1991).
19. S. B. Gelvin and L. L. Habeck, *J. Bacteriol.* **172**, 1600 (1990).
20. T. Steck and C. I. Kado, *ibid.*, p. 2191.
21. G. Ziegelin *et al.*, *DNA Sequence—J. DNA Sequencing Mapping* **1**, 303 (1991).
22. P. J. J. Hooykaas, C. Roobol, R. A. Schilperoort, *J. Gen. Microbiol.* **110**, 99 (1979).
23. J. H. Miller, *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1972).
24. L. S. Melchers *et al.*, *EMBO J.* **8**, 1919 (1989).
25. J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).
26. H. C. Birnboim and J. Doly, *Nucleic Acids Res.* **7**, 1513 (1979) (modified by P. Ebert, personal communication).
27. P. R. Hirsch and J. E. Beringer, *Plasmid* **12**, 139 (1984).
28. T. Mozo and P. J. J. Hooykaas, *Plant Mol. Biol.* **16**, 917 (1991).
29. P. J. J. Hooykaas, A. Den Dulk-Ras, R. A. Schilperoort, *Plasmid* **4**, 64 (1980).
30. B. P. Koekman, P. J. J. Hooykaas, R. A. Schilperoort, *ibid.* **7**, 119 (1982).
31. A. Den Dulk-Ras, unpublished strain.
32. P. H. Klapwijk *et al.*, *J. Bacteriol.* **141**, 129 (1980).

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Sparse Population Coding of Faces in the Inferotemporal Cortex

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How does the brain represent objects in the world? A proportion of cells in the temporal cortex of monkeys responds specifically to objects, such as faces, but the type of coding used by these cells is not known. Population analysis of two sets of such cells showed that information is carried at the level of the population and that this information relates, in the anterior inferotemporal cortex, to the physical properties of face stimuli and, in the superior temporal polysensory area, to other aspects of the faces, such as their familiarity. There was often sufficient information in small populations of neurons to identify particular faces. These results suggest that representations of complex stimuli in the higher visual areas may take the form of a sparse population code.

An unresolved issue in cortical neurophysiology is whether the sensory hierarchies eventuate in small numbers of single cells tuned to complex patterns or in large populations of broadly tuned cells. Sparse coding theories suppose that individual cells should show specificity for behaviorally relevant stimuli (*1*), whereas population theories suppose that distributed patterns of activity in neuronal populations underlie perception and behavior and, correspondingly, expect cells to exhibit broadly graded responses (*2*, *3*). In the context of this issue, the specificity of neurons responsive to faces in the inferotemporal cortex (*4*–*7*) has been interpreted as strong support for sparse coding theories. On the other hand, although neurons responsive to faces may be sharply tuned to a class of stimuli, they modulate their firing to more than one stimulus and tend to be broadly tuned to stimuli within the category (*5*).

To address the question of the type of coding evidenced by neurons responsive to faces, we examined 850 unit recordings in the anterior inferotemporal cortex (AIT) and in the anterior superior temporal polysensory area (STP) of macaque monkeys (*Macaca fuscata*) while the monkeys performed a face discrimination task (6). The face discrimination task involved differential response to 3 of the faces from 27 other faces. The monkeys responded at greater than 90% correct performance. We analyzed responsesto the set of 27 faces that met two selection criteria (Fig. 1). The cells were divided into two groups according to whether they were recorded from AIT (41 cells, 26 from monkey A and 15 from monkey B) or in the STP (30 cells, 25 from monkey A and 5 from monkey B). This sample of cells represented 8% of the total number of recorded cells.

To represent quantitatively the population responses to the faces, we applied multidimensional scaling (MDS) to the two populations, a technique that has been used for qualitative analysis of population encoding of complex stimuli (*7*). MDS produces a configuration of points in a small number of dimensions that represent the population responses to the faces. The distances between the points of the MDS configuration are as close as possible to the Euclidean distances between points corresponding to each face response in a high dimensional space whose dimensions are defined by the cells (8). Two-dimensional configurations were derived (Fig. 1), and these explained 70% and 75% of the variance in the AIT and STP data, respectively. These variance-explained statistics were surprisingly high given the sharp dimensional reduction from tens of dimensions (the number of cells in the two populations analyzed) to only two dimensions, and this suggested that the coding of faces in the two populations was redundant. The configurations of points that represented the face stimuli for the AIT and STP populations are shown in Fig. 1, A and B. Faces plotted close together evoked a similar pattern of response across the population, whereas faces plotted far apart evoked very different population responses.

We investigated what characteristics of the faces the AIT and STP cells were coding, en masse. To do this we exploited the fact that the physical properties of the set of faces on which the monkeys performed the face discrimination task had been extensively quantified (Fig. 2A) (6). In addition to the 29 variables that quantified distances between the facial elements, we derived two further models. The first was a "general physical similarity" model, which was computed from the face measurement variables by MDS. A two-dimensional solution (Fig. 2B) explained 94% of the variability in the face measurements, reflecting their redundancy (all the width measurements tend to covary with the general width of the face, for example). The second additional model represented the "familiarity" of the faces, some of which belonged to humans known in varying degrees to the monkeys. Faces of people unknown to the monkeys were coded with a 1, those of people occasionally seen by the monkeys with a 2, those of people more

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