Patient	Age	Sex	Diagnosis	Tx date	Rejection	Current lymphocyte count ($\times 10^9$/L)	Current creatine (μmol/L)
1	54	F	PCK	6-97		0·9	140
2	54	F	PCK	8-97	Day 60. Acute rejection? ×3 steroid 500 mg/day	0·5	116
3	29	M	DM	8-97		0·7	111
4	29	M	DM	9-97		1·2	117
5*	50	M	PCK	10-97		0·3	149
6*	40	M	I	10-97		0·6	136
7	35	F	PCK	10-97		1·5	79
8†	33	M	GN	10-97	Day 35. Acute rejection. ×3 steroid 1 g/day	1·6	250
9	43	F	HTN	10-97		0·4	106
10	35	F	HTN	11-97		1·8	200
11	41	M	I	11-97		1·4	157
12	66	M	GN	11-97		1·6	131
13‡	60	M	PCK	11-97		0·8	407

*Asystolic donor. †Thrombosis of inferior of 3 arteries with ureteric and renal infection. Reconstruction of ureter required. ‡Severe congestive cardiac failure prior to transplantation. PCK=polycystic kidney, DM=diabetes mellitus, HTN=hypertension nephropathy, I=idiopathic, GN=glomerulonephritis, Tx=transplantation.

Patient details

antibody, is a powerful lytic agent for both T and B lymphocytes but not bone-marrow stem cells.[5] We report use of this antibody in low doses given to recipients of cadaveric-kidney allografts.

13 patients with kidney failure, not previously transplanted, received cadaveric renal allografts (two were from asystolic donors) and two doses of 20 mg intravenous campath 1H. Five of the 13 were given the first dose just before surgery and the second dose 24 h later; eight were given the first dose shortly after surgery and the second 24 h later, For all patients, 48 h after the second dose of antibody, cyclosporin (Neoral) was started at a dose aimed to produce maintenance through concentrations 75–125 μg/mL. 500 mg methylprednisolone was given intravenously 30 min before the first dose of campath 1H to minimise reactions to cytokine release. Lymphocyte cytotoxicity tests between donor serum and recipient cells were negative in all cases. HLA mismatches varied from 1 to 5. Standard renal allografting surgical techniques were used. Patients were given routine antibiotic prophylaxis and acyclovir if there was a cytomegalovirus recipient-donor mismatch. Cotrimoxazole was given for 6 months and amphotericin lozenges for 6 weeks.

All 13 patients have sustained function in their allografts between 6 and 11 months after surgery, and 12 are receiving low-dose cyclosporin. One patient, case 8 (table), is taking prednisone 10 mg/day and 100 mg/day azathioprine after a rejection episode. All recipients had immediate and profound depletion of circulating lymphocytes. Counts remained very low for 2–6 months and then recovered quite quickly. Two patients had episodes of impaired renal function. In case 2, the biopsy was not typical of rejection but methylprednisolone was given, along with antimicrobials for *Klebsiella* urinary infection. In case 8, a biopsy specimen confirmed acute cellular rejection, which responded to steroids. There were no serious side-effects despite low lymphocyte counts.

Immunosuppressives may overwhelm the patient's immune system and prevent the development of natural active graft acceptance. Long-term immunosuppression carries the dangers of infection, cancer, renal damage, hypertension, diabetes, hyperlipidaemia, hirsutism, cushingoid facial appearance, and bone necrosis. If maintenance immunosuppression is stopped, rejection usually follows except in some liver-transplant patients.

Strategies to establish tolerance will probably require the initial use of immunosuppressive agents, an induction treatment to prepare the immune system for active graft acceptance by almost, or the Latin equivalent prope, tolerance, and then very low-dose maintenance immunosuppression to guard against rejection.

How many lymphocyte depletion have helped engraftment in our patients? We speculate that the reduced number of cell numbers inhibited the opportunity for T cell to T cell interactions that are required for rejection. In the absence of initial aggression and inflammation, the healed graft may lose its capacity to immunise and may instead behave as a tolerogen.

We are currently studying the in-vitro reactivity of the repopulated lymphocytes in the renal recipients in an attempt to determine how tolerant these graft recipients have become after having received campath 1H. A randomised trial is planned to compare the protocol described in this report with conventional treatment.

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Efficient and persistent gene transfer of AAV-CFTR in maxillary sinus

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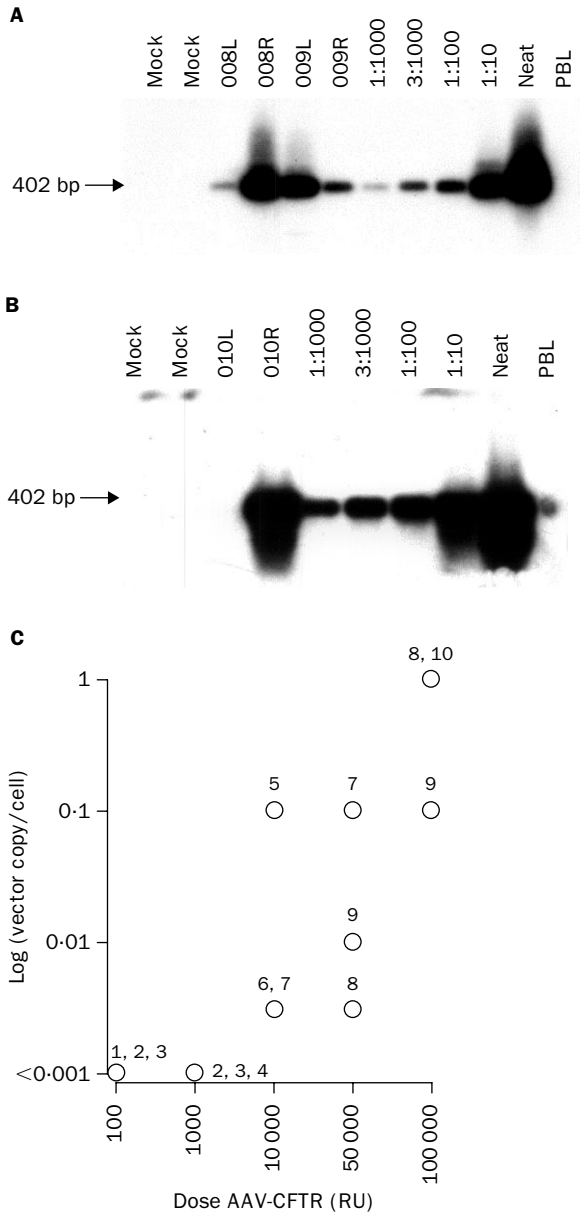
Cystic fibrosis is a common, lethal, genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), and it is an attractive target for gene therapy. Adeno-associated virus (AAV) is a naturally replication-deficient single-stranded DNA parvovirus. Preclinical studies showed that *CFTR* transcripts and protein can be detected up to 6 months after transduction with an AAV-CFTR vector,[1] with no pathogenicity,[2] suggesting that AAV performs well as a gene transfer vehicle for CFTR. The maxillary sinuses are attractive for evaluating new treatments of cystic fibrosis because they have ion-transport systems and microbiology similar to those of the lower respiratory tract.[3,4] Recurrence of maxillary sinusitis may prove to be a surrogate for infectious exacerbations characteristic of cystic fibrosis lung disease.

Ten patients who had undergone bilateral endoscopic antrostomies were treated with AAV-CFTR[2] in a phase I, randomised, non-blinded, dose escalation protocol.[5] Five patients received one dose of vector and five were treated with an initial low dose followed by a second, higher dose in the contralateral maxillary sinus. The figure reveals semiquantitative PCR of sinus biopsies from patients treated with 100 000 replication units of AAV-CFTR. At this dose, DNA transfer was observed at 0·1–1 vector copy per cell in biopsies done 14 days after treatment. Persistent DNA transfer was noted in patients 8 and 9, both of whom



Semiquantitative PCR revealing vector DNA in maxillary sinus biopsy samples 13–70 days after treatment

Bilateral maxillary sinus biopsy done 14 (SD 1) days after last treatment.

(A) is Southern blot of amplified DNA from patients 8 and 9. Patient 8 had had left-sided treatment with 50 000 RU 70 days earlier and right-sided treatment with 100 000 RU 14 days before biopsy. Patient 9 received 100 000 RU (left) and 50 000 RU (right) 13 and 42 days before biopsy, respectively.

(B) Patient 10 had had right-sided treatment with 100 000 RU 14 days earlier, left side not treated.

Vector standards consist of hela cells containing single integrated copy of AAV-CFTR spiked into wild-type hela cells at ratios shown. Assay is specific for vector-derived sequences and does not detect endogenous CFTR. Sensitivity is 1 copy per 1000 cell equivalents DNA and is semiquantitative up to 1 copy per cell (neat).

(C) Plot of all day 14 PCR results, showing gene transfer in all patients treated with 10 000 RU or more. Superscripts show patient numbers.

were previously treated with 50 000 RU of AAV-CFTR 70 and 41 days earlier, respectively (longest intervals tested). Patient 10 received only one dose of AAV-CFTR and the contralateral, untreated sinus remains free of gene transfer (figure B). All patients treated with doses of 10 000 units or more exhibited detectable gene transfer (C).

No evidence of vector transcripts was obtained by reverse transcriptase PCR after AAV-CFTR treatments, but a major difficulty with PCR in this setting is discrimination between vector DNA and vector RNA; no sequence present in the vector mRNA is not also present in vector DNA. An alternative measure of expression, transepithelial potential differences,[5] may be useful. The baseline potential difference is −45·5 (SD 15·1) mV, which depolarises upon addition of amiloride (by 57·5 [26·5]% in 72 previously untreated sinuses). There is no response to isoproterenol in previously untreated sinuses, as expected in cystic fibrosis. All six sinuses treated with 50 000 and 100 000 units of AAV-CFTR exhibited by day 14 hyperpolarisations in response to superfusion with isoproterenol, amiloride, and low-chloride-containing solutions, as would be expected if functional CFTR is being expressed. Similar results were observed at day 7 but isoproterenol-induced hyperpolarisations were absent by day 28.

Vector administration did not change the endoscopic appearance of the maxillary sinuses or the acute inflammation present in maxillary sinus biopsies at day 14. All patients had detectable levels of antibodies to AAV capsid, but no consistent change in antibodies was seen after treatment with AAV-CFTR. Eight serious adverse events were observed in four patients; there were six subacute pulmonary deteriorations requiring hospital admission and intravenous antibiotics (typical for cystic fibrosis patients), one patient required revision sinus surgery, and one had a recurrent episode of cholelithiasis requiring medical treatment. Adverse events did not seem to be related to AAV-CFTR.

This phase I study suggests that AAV-CFTR administration to the maxillary sinus results in safe, successful, dose-dependent gene transfer to the maxillary sinus and alterations in sinus potential suggestive of a functional effect, with little or no host immune response. AAV vectors may prove useful for CFTR gene transfer and other in-vivo gene-transfer therapies.

Anna H Messner, Richard Daifuku, Keisuke Kouyama, Julie K Desch, Sara Manley, Alexander M Norbash, Steven Kang, Carol K Conrad, Sandra Friborg, William B Guggino, Barrie J Carter, Julie Maginot, Gerald Berry, Ann Harkins, Zoe Davis, Ilynn Nepomuceno, Sharon Geaghan, and Yao-Pi Hsu made significant contributions to this study. AAV-CFTR was provided by Targeted Genetics Corp. JAW was supported by a Walter Berry fellowship and a Harry Shwachman clinical investigator award. This work was supported by NIH M01 RR00070, CFRI, CFF, and the Berger-Raynolds Fund.

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