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Search for Cross-Species Transmission of Porcine Endogenous Retrovirus in Patients Treated with Living Pig Tissue

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Pig organs may offer a solution to the shortage of human donor organs for transplantation, but concerns remain about possible cross-species transmission of porcine endogenous retrovirus (PERV). Samples were collected from 160 patients who had been treated with various living pig tissues up to 12 years earlier. Reverse transcription–polymerase chain reaction (RT-PCR) and protein immunoblot analyses were performed on serum from all 160 patients. No viremia was detected in any patient. Peripheral blood mononuclear cells from 159 of the patients were analyzed by PCR using PERV-specific primers. No PERV infection was detected in any of the patients from whom sufficient DNA was extracted to allow complete PCR analysis (97 percent of the patients). Persistent microchimerism (presence of donor cells in the recipient) was observed in 23 patients for up to 8.5 years.

Hundreds of patients have been exposed to living porcine cells and tissues for a variety of experimental therapeutic indications, including pancreatic islet cells, skin, and whole livers and spleens for extracorporeal blood perfusion (*1–4*). Proposed future therapeutic indications include solid organ transplantation from transgenic pigs as a means of alleviating the human donor organ shortage (*5–7*). Although most potential pathogens can be eliminated from source animals for xenotransplantation, one that remains is the porcine endogenous retrovirus (PERV), a C-type retrovirus that is permanently integrated in the pig genome (*8–13*). Recent coculture and infectivity experiments have shown that PERV released from pig kidney cell lines, from mitogenically activated porcine peripheral blood mononuclear cells (PBMCs), or from porcine endothelial cells can infect human cells and cell lines in vitro, raising concerns about the possibility of cross-species infection after xenotransplantation (*11, 14–16*).

To supplement the data generated in previous studies (*1, 17*) and to further investigate the potential transmission of PERV to humans, we retrospectively collected PBMCs and serum from patients who had been treated with living pig tissue. The samples were analyzed in multiple laboratories using recently developed and validated assays specific for PERV: PCR (for PERV DNA), RT-PCR (for PERV RNA, a marker for virions), and protein immunoblot antibody assays (for exposure to PERV antigens). Testing strategies were also devised to distinguish whether any PERV DNA detected was due to actual infection or to the presence of pig cells. This could result from a situation analogous to that frequently observed after allotransplantation, termed microchimerism, where cells from the donor organ are known to traffic through the recipient (*18*).

Study participants and testing laboratories. A total of 160 patients (83 males and 77 females, aged 2 to 77 years) participated in

this study (Table 1). One of the following treatments had been used in these patients:

1) Extracorporeal splenic perfusion (ECSP) (n = 100) through spleens from healthy slaughterhouse pigs as "immunotherapy" for various indications. One patient subsequently received chemotherapy (St. Petersburg, Russia).

2) Extracorporeal perfusion for liver failure with the HepatAssist device (Circe Biomedical) (n = 28), which contains pig hepatocytes enclosed in a semipermeable membrane (*19*). After the procedure, 25 of these patients received liver transplants and then pharmacological immunosuppression (Villejuif, France; Los Angeles, USA; Jerusalem, Tel Aviv, and Haifa, Israel).

3) Pig skin grafts (n = 15) for burns (Bochum, Germany).

4) Porcine pancreatic islet cell transplants for diabetes (n = 14). Specimens collected from eight of these patients at different time points were tested previously for evidence of cross-species transmission of PERV (*1*). Nine of these patients also received a kidney allotransplant and pharmacological immunosuppression. Evidence of porcine C-peptide (released from islets) was detected in the urine of four patients for 257 to 460 days after xenotransplant (Huddinge, Sweden; Auckland, New Zealand).

5) Extracorporeal pig kidney perfusion (n = 2). Samples collected from these patients (*20*) at different time points have been previously analyzed (*17*) (Göteborg, Sweden).

6) Extracorporeal perfusion through a whole pig liver (n = 1) followed by a liver allotransplant and pharmacological immunosuppression (Montreal, Canada).

Tests were conducted at four laboratories: Genetic Therapy Inc. (GTI, Gaithersburg, MD), Q-One Biotech Ltd. (Glasgow, Scotland), Primedica Corporation (Rockville, MD), and the Centers for Disease Control and Prevention (CDC, Atlanta, GA). A spectrum of tests was used to detect all possible types of infection: latent, persistent, sequestered, and cleared or "recovered." The testing sites used different primers, antigens, and methodologies in their respective assays.

At GTI, PBMCs were tested for PERV and pig-specific centromeric sequences by PCR, and serum was tested for PERV RNA by RT-PCR. At Q-One, serum was tested for antibodies to PERV by protein immunoblot analysis, and saliva was tested for PERV RNA by RT-PCR. At Primedica, PBMCs from the 28 patients treated with the Hepat Assist device were tested by PCR. Confirmatory PCR and protein immunoblot analyses were performed at the CDC facility.

Study design and testing strategy. The institutional review boards or ethics committees at each participating hospital approved the study. After informed consent specific for the present study was obtained, data were collected regarding dietary or occupational exposure to pig tissue, signs or symptoms that might indicate infection of unexplained etiology, and details of pharmacological immunosuppression. PBMC and serum samples were prepared and aliquoted at the different hospitals and stored at −70°C until dispatched on dry ice to the testing laboratories.

At GTI, Primedica, and CDC, PBMCs were tested for PERV DNA sequences by PCR. A negative result was reported as "no infection detected." However, if PERV DNA was detected, a second PCR was performed to assess whether the sample contained pig-specific sequences (centromeric at GTI and mitochondrial at CDC) indicating the presence of pig cells. If no pig-specific sequences were detected, the patient was reported as "infected." In contrast, if porcine-specific DNA was detected, it was reported as "microchimerism," although PERV infection could not be excluded.

As a way to distinguish between infection and microchimerism in samples with quantitative results, the ratio of PERV to centromeric sequences was calculated and compared to that found in 43 pigs. Theoretically, this ratio would be increased in a patient with infection. Conversely, a ratio in a patient sample that did not significantly exceed the mean ratio found in pigs would suggest that the PERV DNA was entirely due to the presence of circulating pig cells.

GTI tested serum for the presence of circulating virion-associated RNA by RT-PCR. Negative results were reported as "no infection detected." Q-One and CDC tested serum by protein immunoblot analysis for the presence of antibodies to Gag p30; results were reported as "seroreactive" or "seronegative."

Feline leukemia virus (FeLV), another C-type retrovirus, can be sequestered in the salivary gland of infected cats, and the virus is shed in large quantities in saliva (*21*). Thus, where possible, RT-PCR was used at Q-One to test for the presence of PERV virions in the saliva from seroreactive patients or in patients who were found to have microchimerism at GTI and in whom concomitant infection could not be excluded. After written informed consent was obtained, blood and saliva were also collected from the close contacts (people living in the same household) of seroreactive patients detected at Q-one to test for evidence of potential horizontal human-to-human transmission of PERV.

Detection of PERV DNA by PCR. GTI performed a quantitative PCR assay to test for PERV DNA, using a fluorescence-based real-time PCR method in which DNA extracted from PBMCs (*22*) was tested using PERV *pol* primers and a *pol* probe (*23*), conserved for four published PERV variants (*10–13*). Validation showed that the limit of detection was one copy of PERV per 3.3 μg of total DNA (500,000 cells). The sensitivity of the assay was defined as 10 copies per 500,000 cells, which gave >99.99% confidence of detecting ≥10 copies (that is, <0.01% chance of a false negative). On the basis of a total of 405 control DNA samples, the false positive rate for this assay was 9.88%. The samples were therefore retested up to two additional times to confirm a pos-

Table 1. Patient demographics.

Procedure	N	Age (years)	Duration of exposure	Months since treatment ± SD (range)
Extracorporeal liver perfusion	1	59	4.25 hours	40.5
Extracorporeal kidney perfusion	2	40 to 50	15 and 65 min	33.9 ± 2.8 (31.9 to 35.8)
Bioartificial liver perfusion	28	11 to 65	11.75 hours (2 to 30 hours)	25.8 ± 18.7 (2.4 to 60.8)
Pancreatic islet cells	14	19 to 59	1 to 460 days	59.7 ± 24.2 (18.8 to 92.9)
Skin	15	8 to 67	10 days (estimated average)	101.9 ± 34.4 (35.7 to 149.5)
Extracorporeal splenic perfusion	100	2 to 77	50 to 60 min	29.7 ± 28.5 (0 to 102)
Total	160	2 to 77		38.5 ± 34.8 (0 to 149.5)

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itive PCR result, and only samples that tested positive in all three assays were reported as positive. This testing algorithm reduced the false positive rate to <0.1%, with no significant increase in the probability of obtaining false negative results (<0.03%). The PERV PCR assay was quantitative, with a linear range of 10 to 10^6 copies (*24*). Positive samples with fewer than 10 copies could not be quantitated.

At CDC, DNA was tested using primers for human β-actin to assess whether the DNA extracted from PBMCs was suitable for amplification. PCR was then performed on β-actin–positive samples using primers for conserved sequences of the *gag* and *pol* PERV genes. The amplified PCR products were hybridized with ^{32}P end-labeled oligonucleotide probes specific for *gag* or *pol* (*25*). The sensitivities of these assays were determined by performing serial dilutions of plasmids containing PERV *gag* or *pol*. Validation showed that the limit of detection of the PCR assay for *gag* and *pol* was one copy and five copies, respectively, per 1 μg of DNA (150,000 human cells). The sensitivity of both assays was defined as 10 copies per 150,000 cells, which gave >99.99% confidence of detecting ≥10 copies (<0.01% chance of a false negative). On the basis of empirical data from 69 negative samples, the false positive rate was <1/69 (1.45%). Positive samples were therefore retested and were reported as positive only if the initial result was confirmed (false positive rate <0.021%). If the results for either the *gag* or *pol* PCR assays were positive, the sample was considered positive for PERV DNA. As determined by empirical data from 70 control samples containing pig cell DNA lysates at the limits of detection, the false negative rate was 0.02% for duplicate testing with *gag* and 0.18% for duplicate testing with *pol*.

At Primedica, PCR was performed with primers designed to amplify the *pol* gene of PERV (*26*). The PCR products were detected by Southern (DNA) blot hybridization and a fluorescein-tagged oligonucleotide probe. The sensitivity of the assay was assessed by performing serial dilutions with known numbers of porcine (PK-15) cells; the results showed that the sensitivity of the assays was 0.3 of a PK-15 cell per 10^5 cells, with an exception for one sample, where the sensitivity of the assay was 1.0 PK-15 cell per 10^5 cells.

Distinguishing PERV infection from microchimerism. PERV-positive DNA samples were also tested by PCR for the presence of porcine-specific sequences. The GTI centromeric PCR assay had a limit of detection of 10 copies per 500,000 cells and was capable of quantifying levels of centromeric sequences ranging from 10 to 10^6 copies (*27*). This assay could not discriminate between a negative sample and one containing fewer than 10 copies; therefore, any sample with a value of less than 10 was considered to be negative. A positive test was considered as indicative of circulating pig cells. Samples were analyzed in triplicate where possible and reported individually. Additional testing was performed that shows that infection could be detected in the presence of microchimerism when >0.01% of the human cells in a sample containing 10^7 human and 20 pig cells are infected.

The sensitivity of the mitochondrial PCR assay performed at CDC was one copy per 150,000 human cells (*25*). Samples positive for PERV DNA and negative for porcine mitochondrial sequences would be interpreted as infected. The presence of PERV and mitochondrial sequences would indicate the presence of microchimerism. As the assay was not quantitative, the possibility of PERV infection coexisting with microchimerism could not be excluded.

Results of PCR testing. Using the most conservative approach, positive results from any laboratory resulted in an overall positive interpretation (Table 2). Five of the initial 160 samples (3%) yielded insufficient DNA for analysis. Of the remaining samples, 125 (81%) were negative for PERV DNA. The remaining 30 patients positive for PERV DNA had all been treated with ECSP. Of these, 23 showed clear evidence of circulating pig DNA (centromeric or mitochondrial or both), indicating microchimerism. The remaining seven did not have sufficient DNA to allow testing for pig-specific sequences, and thus the infection status of these patients was uninterpretable.

In 19 of the 23 patients with microchimerism, the PERV copy number could not be quantitated, as there were <10 copies per 500,000 cells. For two of the four patients with quantitative PERV DNA results, there was insufficient DNA in the same aliquot to be able to quantitate the number of centromeric sequences. Thus another aliquot was used for quantitation, which precluded the calculation of a ratio for these patients. The PERV:centromeric ratios were calculable from the same aliquot on patients 6017 (24/1300 = 0.0185) and 6100 (21,600/690,000 = 0.0313). No statistical difference was observed when these values were compared to those obtained from a cohort of 43 Large White pigs (mean ± SD = 0.0416 ± 0.0411, upper 95% confidence interval = 0.125), even when all PERV and centromeric values were combined for all aliquots for each patient. Hence, the data suggest that these patients were not infected.

Seven months after the initial blood sampling, another PBMC sample was collected from selected patients. These included four of the five patients with insufficient DNA for analysis, four of the seven patients whose results were initially uninterpretable, and 12 of the 23 patients with microchimerism. All but one of these second time-point samples were negative; the remaining sample had insufficient DNA to test for porcine sequences (patient 6029). Three of the seven patients with initially uninterpretable infection status due to insufficient DNA could not be resampled (patients 6095, 6004, and 6010). For patient 6095, from whom blood was taken on the same day as the splenic perfusion, microchimerism is almost certain because pig cells are flushed from the spleen into the patient's circulation during treatment. Patients 6004 and 6010 tested negative at GTI but were positive at CDC, implying a very low level of PERV DNA in the sample.

RT-PCR testing for PERV RNA. At GTI, RNA extracted from serum using the QIAamp viral RNA kit (Qiagen) was reverse-transcribed, amplified, and detected by means of primers and probes complementary to conserved regions of the PERV *pol* gene (*28*). Validation of this assay showed 100% detection of PERV RNA in 96 serum samples spiked with 400 virus-like particles (VLPs) per milliliter, as counted by transmission electron microscopy; thus, the limit of detection for this assay was 400 VLPs/ml. Positive results were confirmed by retesting.

At Q-One, RNA extracted from saliva using the QIAamp viral RNA kit was reverse-transcribed, amplified, and detected by means of primers and probes derived from the PERV *gag* gene (*29*). Validation of the assay showed that the limit of detection was 100 VLPs/ml and that it could consistently detect 1000 VLPs/ml.

No PERV RNA was detected in the serum of any of the 160 patients, nor in saliva from the two seroreactive ECSP patients (6052 and 6062, see below), their five close contacts, and 16 ECSP patients with microchimerism.

Detection of antibodies to PERV. At Q-One, a recombinant PERV p30 Gag protein (PERV B1 Gag sequence expressed in *Escherichia coli*) was used as an initial screen for antibodies to PERV, and positive samples were then tested against the purified whole virus (isolated from a PERV-positive human 293 cell line). The specificity of the assay was assessed by testing a series of serum samples from patients with no known treatment with living porcine material (*30*). A sample was considered to be positive when it reacted against p30 Gag antigen in both assays. At CDC, reactivity to Gag and p27 were measured in a protein immunoblot assay with whole-cell lysates prepared from PERV-infected human 293 cells (*31*).

No antibodies to PERV were detected in 156 of 160 patients at either laboratory. Seroreactivity was detected in two patient samples (6052 and 6062) when tested at Q-One; one of these (6052) had a weak signal and tested negative 7 months later. However, all these samples were found to be seronegative by CDC, using any combination of Q-One and CDC antigens and methods (Table 3).

The five close contacts of these two patients were seronegative when tested at Q-One.

A weak seroreactivity was detected in two patient samples (10018 and 8004) only when tested at CDC (Table 3). Similar reactivity was present in serum samples obtained 1 and 3 days after their respective procedures, suggesting preexisting antibodies rather than seroconversion. None of the four seroreactive patients from either laboratory had molecular evidence that would support PERV infection.

Unexplained symptoms and signs. Three patients who had extracorporeal perfusion with the HepatAssist device reported skin rashes of unclear etiology. Two of these rashes have persisted, and one of these patients also reported a 4-day febrile episode of unknown origin. One patient who received a porcine skin graft reported an ongoing skin rash. All tests for PERV were negative in these patients. No other clinically significant symptoms or events were reported, even in those patients found to exhibit microchimerism.

Summary and interpretation. No evidence of persistent PERV infection could be detected in any of the patients (97%) in whom sufficient DNA was available to allow PERV PCR and (if necessary) to allow amplification of porcine sequences to be performed. This includes 36 patients who were pharmacologically immunosuppressed and therefore presumed to be at increased risk of infection. Similar negative data, drawn from comparable assays, have been reported for samples collected at different time points from eight of the patients who received pancreatic islets in Sweden and the two patients who underwent extracorporeal swine kidney perfusion (*1, 17*).

Table 2. Detailed results from the Russian patient samples in which PERV DNA was detected. QNS, quantity not sufficient; +, positive; −, negative; NT, not tested. Results were classed as uninterpretable when there was insufficient DNA to complete the testing algorithm.

Patient ID	Months since procedure	CDC				GTI		Overall interpretation
		PERV *gag*	PERV *pol*	PERV result	Pig mito*	PERV result†	Pig centromeric result*†	
6082	0	−	+	+	+	+ (<10, <10, <10)	+ (22,−,−)	Microchimerism
	6.4	−	−	−		−		No infection detected
6098	0	+	−	+	+	+ (12, <10, <10)	+ (420, 340, 740)	Microchimerism
	6.2	−	−	−		−		No infection detected
6100	0	+	+	+	+	+ (4600, 3600, 21,600)	+ (690,000)	Microchimerism
	6.5	−	−	−		−		No infection detected
6027	0	−	+	+	+	−		Microchimerism
6074	0	−	+	+	+	−		Microchimerism
6089	0	+	−	+	+	−		Microchimerism
	6.6	−	−	−		NT		No infection detected
6095	0	−	−	−		+ (<10, <10, <10)	QNS	Uninterpretable
6029	0	−	+	+	+	+ (<10, <10, <10)	+ (48, 45, 27)	Microchimerism
	6.8	−	−	−		+ (<10, <10, <10)	QNS	Uninterpretable
6026	0.2	+	−	+	+	−		Microchimerism
	6.7	−	−	−			NT	No infection detected
6007	0.2	+	+	+	QNS	QNS		Uninterpretable
	7.2	−	−	−		−		No infection detected
6028	0.4	−	+	+	+	+ (<10, <10, <10)	+ (31, 98, 13)	Microchimerism
	7.2	−	−	−		−		No infection detected
6032	0.4	+	+	+	+	−		Microchimerism
6006	0.5			QNS		+ (<10, 11, <10)	+ (405, 123)	Microchimerism
6030	1.1			QNS		+ (<10, <10, <10)	+ (856)	Microchimerism
6041	14.8	−	+	+	+	−		Microchimerism
6035	14.8			QNS		+ (11, <10, <10)	QNS	Uninterpretable
	21.5	−	−	−		−		No infection detected
6011	16.4	+	−	+	+	−		Microchimerism
	23.1	−	−	−		NT		No infection detected
6013	26.0	−	+	+	+	−		Microchimerism
6004	34.5	+	+	+	QNS	−		Uninterpretable
6024	34.7			QNS		QNS‡	+ (263, 59, 54)	Microchimerism
6002	36.9			QNS		+ (<10, <10, <10)	+ (500, 1800)	Microchimerism
	43.8	−	−	−		−		No infection detected
6016	37.8	+	+	+	+	−		Microchimerism
6017	41.7			QNS		+ (24, 10, <10)	+ (200, 1300)	Microchimerism
	49.1	−	−	−		−		No infection detected
6034	49.0	+	−	+	QNS	QNS		Uninterpretable
	55.5	−	−	−		−		No infection detected
6022	49.1			QNS		+ (<10, <10, <10)	+ (170, 95, 210)	Microchimerism
	56.1	−	−	−		−		No infection detected
6010	73.4	−	+	+	QNS	−		Uninterpretable
6023	73.7			QNS		+ (<10, <10, <10)	+ (190, 58)	Microchimerism
6003	88.8	+	+	+	QNS	−		Uninterpretable
	96.1	−	−	−		NT		No infection detected
6005	89.0	−	−	−		+ (<10, <10, <10)	+ (190, 160)	Microchimerism
	95.7	−	−	−		−		No infection detected
6012	101.4	−	+	+	+	−		Microchimerism

*Tests for porcine mitochondrial DNA or centromeric sequence were only performed in samples testing positive for PERV DNA. †Results are followed in parentheses by individual results per 500,000 cells; results may come from different aliquots. ‡Insufficient sample to complete testing algorithm, PERV PCR performed twice (copy number <10, <10); remaining DNA tested for centromeric sequences.

The absence of identifiable adverse events in any of the 23 patients in whom microchimerism was detected despite 43.7 person-years of cumulative exposure to pig cells is reassuring. The presence of cells of donor origin in sites outside the donor organ has been well described in human allotransplantation when patients are continuously pharmacologically immunosuppressed (*18*). The long-term persistence of microchimerism in patients tested between 2 and 102 months after ECSP was an unexpected finding, as none of these patients have been immunosuppressed since the procedure (Fig. 1). It is possible that these pig cells are dendritic cells, as observed in allotransplantation, or stem cells originating from the pig spleen, which may express low levels of the xenoantigen Galα1-3Galβ1-4GlcNac-R, allowing them to escape antibody-mediated clearance.

The quantitative results obtained from analysis of the patient samples suggest that the level of microchimerism was extremely low (less than one pig cell per 500,000 PBMCs in the majority of cases). This approaches the limit of detection, as determined by performing serial dilutions of plasmid containing viral DNA, of the PERV PCR assays used by the CDC (one copy of *gag* and five copies of *pol* per 150,000 human cells) and by GTI (1 copy of *pol* per 500,000 human cells) and may explain the differences in PCR results between the two testing sites (Table 2). As predicted by the Poisson distribution, aliquoting of samples containing very few targets may lead to discrepant results. In addition, if particular PERV variants are preferentially amplified because of subtle mismatches in primer sequences, this may also account for the different results reported both between and within laboratories.

Molecular testing results for four PERV-positive patients remained uninterpretable because the amount of DNA was insufficient to rule out the presence of microchimerism. However, considering that PERV DNA was detected in only one of two testing laboratories, the absence of either circulating PERV virions or antibodies to PERV suggests that these detected PERV sequences represent low-level microchimerism rather than infection.

Despite the prolonged presence of pig cells in the circulation of at least 23 of the study patients, no evidence of productive infection was detected by testing either serum (160 patients) or saliva (12 microchimeric patients). These observations are very encouraging, given that the development of disease and transmission of FeLV is usually associated with a persistent plasma viremia and widespread replication of the virus in epithelial cells, hematopoietic cells, and other tissues (*21*).

Only two of 160 patients were seroreactive at Q-One. Because preprocedural serum was not available from these two patients, it was not possible to assess whether these antibodies were present before treatment. Both were seronegative at CDC, and a substantial effort was made to resolve these differences (Table 3). The discrepant results do not appear to be antigen dependent, but are more likely to be due to technical differences between the two methodologies. It is not yet possible to determine whether the antibody response seen at Q-One represents an immunological response to PERV protein or whether it is due to cross-reactivity with an unrelated antigen. Cross-reactive antibodies to retroviral Gag proteins have been described (*32–34*). All other molecular tests (PCR, RT-PCR in serum and saliva) in these patients were negative, and the close contacts of these patients were also found to be negative for PERV by PCR, as well as seronegative by protein immunoblot.

All retrospective studies are limited in their ability to identify the true incidence of infection or associated clinical symptoms. As has been suggested (*35, 36*), closely monitored prospective trials would allow a more comprehensive evaluation of the possibility of cross-species transmission of PERV. Prospective clinical trials would also allow comparison of the PERV: centromeric ratio for a given patient to that of the specific source pig, thereby increasing the reliability with which infection could be distinguished from microchimerism.

We have not detected any conclusive evidence of cross-species or human-to-human transmission of PERV. These results support the use of closely monitored clinical trials as an approach to assessing the safety and efficacy of using porcine cells, tissues, or organs therapeutically in humans.

Table 3. Interlaboratory testing of samples with discrepant serologic results, performed to resolve discrepancies (+, positive; −, negative).

Patient ID*	Time since procedure	Testing site	Antigen and method	Result#
6062	4.6 months	Q-One	p30, virus†	+
		CDC	Infected 293‡	−
		CDC	p30§**	−
		Q-One	Infected 293‖	+
		Q-One	p30¶	Weak +
	11.6 months	Q-One	p30, virus†	+
		CDC	Infected 293‡	−
6052	73.2 months	Q-One	p30, virus†	Weak +
		CDC	Infected 293‡	−
		Q-One	Infected 293‖	−
		Q-One	p30, virus†	−
		CDC	p30, virus§	−
	80.2 months	Q-One	p30, virus†	−
		CDC	Infected 293‡	−
		CDC	p30, virus‡	−
8004	3 days	CDC	Infected 293‡	Weak +
	72.1 months	CDC	Infected 293‡	Weak +
		Q-One	p30, virus†	−
10018	1 day	CDC	Infected 293‡	Weak +
	58.9 months	CDC	Infected 293‡	Weak +
		Q-One	p30, virus†	−

*ID codes: 6xxx, Russia (ECSP); 8xxx, Sweden (pancreatic islet cells); 10xxx, USA (HepatAssist device). †Q-One method as described. ‡CDC method as described. §Q-One antigen, but using CDC methodology. ‖CDC antigens, but using Q-One methodology. ¶Q-One p30 antigen, but using modified Q-One methodology (more stringent washes and altered incubation conditions). #Repeat testing samples were repeatedly frozen and thawed. **Q-One antigen, but using modified CDC methodology (more stringent washes and extended incubation).

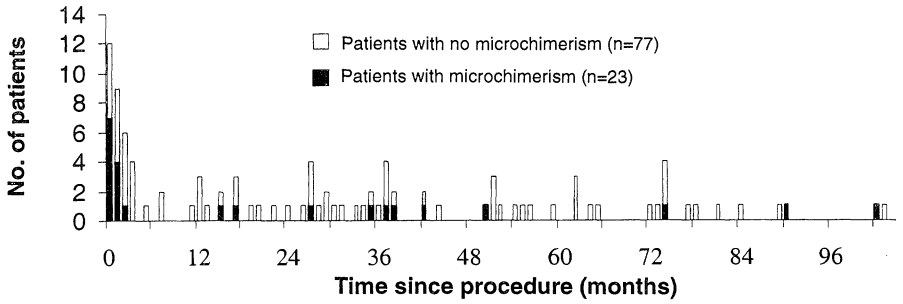

Fig. 1. Proportion of patients exhibiting microchimerism at the time of sampling in relation to time elapsed since ECSP. Bars represent the total number of patients; solid bars represent the proportion of patients found to have microchimerism.

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22. At GTI, DNA was isolated from PBMCs using a genomic DNA isolation procedure (QIAamp Blood Kit, Qiagen).
23. DNA (3.3 μg) was amplified by PCR with primers 5′-AGCTCCGGGAGGCCTACTC-3′ and 5′-ACAGCCGTTGGTGTGGTCA-3′, probe 5′-FAM-CCACCGTGCAGGAAACCTCGAGACT-TAMRA-3′ [where FAM (6-carboxyfluorescein) is a fluorescent tag and TAMRA (6-carboxytetramethylrhodamine) is a fluorescence quencher], and the 7700 sequence detector system (ABI/Perkin-Elmer). The PCR was performed in a 100-μl reaction volume containing 5 U of Taq polymerase and 1 U of UNG, 300 nM each primer, and 100 nM probe in the Universal Master Mix buffer (Perkin-Elmer). The cycling parameters were 2 min at 50°C, 10 min at 95°C, and 60 cycles of 15 s at 95°C and 1.5 min at 60°C.
24. A total of 10, 10^2, 10^3, 10^4, 10^5, and 10^6 copies of the *pol*/*env* plasmid in a background of 3.3 μg of DNA from three species were used to prepare standard PERV *pol* curves. The validation consisted of three runs for each species, each of which included five PERV *pol* standard curves, five reagent controls, 45 negative controls, and 10 one-copy positive controls. Validation showed that the assay could detect one copy of PERV in a background of 500,000 human cells and that it was quantitative over a range of 10 to 10^6 copies of PERV.
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26. DNA (0.66 μg) was amplified using primers for the conserved region of the *pol* gene. Primers used were 5′-GCTACAACCATTAGGAAAACTAAAAG-3′ and 5′-AACCAGGACTGTATATCTTGATCAG-3′.
27. DNA (3.3 μg) was amplified by quantitative PCR with primers 5′-TAGCCATGCTGCATGTAATGC-3′ and 5′-GGAGCGTGGCCCAAT-3′, probe 5′-FAM-ATGCTGCATGGAATGCACTACCTTCAA-TAMRA-3′, and the 7700 sequence detector system (ABI/Perkin-Elmer). The PCR was performed in a 100-μl reaction volume containing 5 U of Taq polymerase and 1 U of UNG, 300 nM each primer, and 100 nM probe in 1× Universal Master Mix (Perkin-Elmer). The cycling parameters were 2 min at 50°C, 10 min at 95°C, and 40 cycles of 15 s at 95°C and 1.5 min at 60°C.
28. RNA was reverse-transcribed with a primer containing a 19-base sequence at the 3′-end specific for PERV *pol* and a 30-base tag sequence at the 5′-end, 5′-GAACATCGATGACAAGCTTAGGTATCGATAACAGCCGTTGGTGTGGTCA-3′, for 30 min at 42°C using 50 U of MuLV reverse transcriptase (RT) followed by 10 min at 90°C. Control reactions with no RT were also performed as a control for contamination with PERV genomic DNA. After reverse transcription, the cDNA was amplified with primers specific for PERV *pol* (5′-AGCTCCGGGAGGCCTACTC-3′) and the cDNA specific 30-base tag primer (5′-GAACATCGATGACAAGCTTAGGTATCGATA-3′) and probe 5′-FAM-CCACCGTGCAGGAAACCTCGAGACT-TAMRA-3′. Amplification was detected with the 7700 sequence detection system (ABI/Perkin-Elmer). The cycling parameters were 2 min at 50°C, 10 min at 95°C, and 42 cycles of 15 s at 95°C and 2 min at 65°C.
29. At Q-One, RNA was reverse-transcribed for 50 min at 42°C by means of 5 U of Moloney murine leukemia virus (MuLV) reverse transcriptase (RT) with random hexanucleotides. After transcription, ribonuclease H was added to the reaction mix, which was then heated to 37°C for 15 min. Control reactions with no RT were also performed as a control for contamination with PERV genomic DNA. After reverse transcription, 5 μl of the sample was amplified using primers from within the PERV *gag* gene, 5′-GCGACCCACGCAGTTGCATA-3′ and 5′-CAGTTCCTTGCCCAGTGTCCTT-3′. A second nested PCR assay was carried out using the primers 5′-TGATCTAGTGAGAGAGGCAGAG-3′ and 5′-CGCACACTGGTCCTTGTCG-3′. RT-PCR products were analyzed by agarose gel electrophoresis.
30. Purified recombinant PERV protein or virions were treated with SDS/β-mercaptoethanol and separated by electrophoresis through a tris-glycine acrylamide gel (12%) before immunoelectrotransfer onto PVDF membrane (Imobilon) (200 mA, 1 to 2 hours). The membrane was washed in phosphate-buffered saline (PBS)/Tween before being treated with milk protein. Test sera were diluted to 1:200 in PBS/Tween containing 5% milk protein and incubated with membranes for 1 to 2 hours at room temperature. Blots were washed in PBS/Tween and incubated with the secondary antibody, an alkaline phosphatase-labeled antiserum diluted at 1:1000, for 1 hour at room temperature. After incubation, the membrane was washed repeatedly in PBS/Tween and developed with 5-bromo-4-chloro-2-indoyl phosphate/nitro blue tetrazolium, which gives a dark blue precipitate when labeled antibody has bound. Negative controls were performed by incubating membranes with serum from individuals who had not been exposed to porcine tissues. The protein immunoblot assay was validated with serum samples from a variety of sources (200 healthy humans, 58 HIV-1–positive humans, 18 HIV-2–positive humans, 13 HTLV–positive humans, four butchers with lymphoma, 20 transplant patients, and 10 CMV-positive humans with transplants).
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Superconductivity and the *c* Axis Spectral Weight of High-T_c Superconductors

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The temperature dependence of the *c* axis spectral weight (frequency integral of the interplane conductivity) of high transition temperature (high-T_c) superconductors is shown to be a probe of thermal and quantal fluctuations of the phase of the superconducting order parameter. The behavior of underdoped cuprates is shown to be a natural consequence of superconducting pairing without long-ranged phase coherence. Very underdoped cuprates are found to have strong phase fluctuations, even for temperatures much less than the transition temperature.

The frequency (ω) and temperature (T) dependence of the interplane conductivity σ_c is one of the long-standing mysteries of high-temperature superconductivity. Particular attention has focused on the spectral weight, the integral of the real part of the conductivity over some frequency range, which has been observed (*1–6*) to have a strong temperature dependence in many high-T_c materials. This dependence on T was argued by some to be highly anomalous and to provide strong evidence in favor of the "interlayer tunneling" model of high-temperature superconductivity (*7–10*). Other workers (*11–14*) have argued that some aspects of *c* axis transport can be understood in relatively conventional terms, but have not considered the temperature dependence of the spectral weight. Here, we present a theory of the *c* axis spectral weight, $K_c(T)$, which accounts for the data.

The high-temperature copper-oxide superconductors have a layered crystal structure in which the important structural subunit is the copper-oxide (CuO_2) plane, with interplane (that is, *c* axis) couplings much weaker than in-plane ones. The conductivity in the direction perpendicular to the planes, $\sigma_c(\omega,T)$, is a complex function with real ($\sigma_c^{(1)}$) and imaginary ($\sigma_c^{(2)}$) parts. We denote the spectral weight at frequencies less than Ω by $K(\Omega,T)$;

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