Xenotropic murine leukemia virus–related virus does not pose a risk to blood recipient safety

Roger Y. Dodd, John Hackett Jr, Jeffrey M. Linnen, Kerri Dorsey, Yanyun Wu, Shimian Zou, Xiaoxing Qiu, Priscilla Swanson, Gerald Schochetman, Kui Gao, James M. Carrick, David E. Krysztof, and Susan L. Stramer

BACKGROUND: When xenotropic murine leukemia virus–related virus (XMRV) was first reported in association with chronic fatigue syndrome, it was suggested that it might offer a risk to blood safety. Thus, the prevalence of the virus among blood donors and, if present, its transmissibility by transfusion need to be defined.

STUDY DESIGN AND METHODS: Two populations of routine blood donor samples (1435 and 13,399) were obtained for prevalence evaluations; samples from a linked donor-recipient repository were also evaluated. Samples were tested for the presence of antibodies to XMRV-related recombinant antigens and/or for XMRV RNA, using validated, high-throughput systems.

RESULTS: The presence of antibodies to XMRV could not be confirmed among a total of 17,249 blood donors or recipients (0%; 95% confidence interval [CI], 0%-0.017%); 1763 tested samples were nonreactive for XMRV RNA (0%; 95% CI, 0%-0.17%). Evidence of infection was absent from 109 recipients and 830 evaluable blood samples tested after transfusion of a total of 3741 blood components.

CONCLUSIONS: XMRV and related murine leukemia virus (MLV) markers are not present among a large population of blood donors and evidence of transfusion transmission could not be detected. Thus, these viruses do not currently pose a threat to blood recipient safety and further actions relating to XMRV and MLV are not justified.

Xenotropic murine leukemia virus–related virus (XMRV) was first reported in selected patients with prostate cancer in 2006.[1] Subsequently, it was also reported in 67% of patients with chronic fatigue syndrome (CFS) and among 3.7% of healthy controls.[2] At the same time, it was suggested that this gammaretrovirus might offer a risk to blood safety.[3] A second study then reported the presence of mouse-derived retroviral *gag* sequences representing polytropic murine leukemia viruses (MLVs) among 87% of CFS patients sampled in the 1990s and 6.7% of contemporary blood donor controls.[4] Whether these two studies are mutually supportive is unlikely. In addition, at least 11 other published studies have failed to confirm a relationship between XMRV and/or MLVs and CFS.[5] Recently, there has been an editorial expression of concern[6] regarding the original study[2] demonstrating XMRV in CFS patients and some of the data have been retracted.[7] A similar relationship between XMRV and prostate cancer has not been confirmed, with more than half of the published studies showing no association.[5] Thus, there is

ABBREVIATIONS: ARC = American Red Cross; CFS = chronic fatigue syndrome; CMIA(s) = microparticle-based chemiluminescence immunoassay(s); MLV(s) = murine leukemia virus(-es); S/CO = sample-to-cutoff ratio; SRWG = Scientific Research Working Group; TMA = transcription-mediated amplification; XMRV = xenotropic murine leukemia virus–related virus.

From the American Red Cross Holland Laboratory, Rockville, Maryland; Abbott Diagnostics, Abbott Park, Illinois; Gen-Probe, Inc., San Diego, California; the Yale University School of Medicine, New Haven, Connecticut; and the American Red Cross Scientific Support Office, Gaithersburg, Maryland.

Address reprint requests to: Roger Y. Dodd, PhD, American Red Cross Holland Laboratory, 15601 Crabbs Branch Way, Rockville, MD 20855; e-mail: dodd@usa.redcross.org.

Received for publication September 28, 2011; revision received October 6, 2011, and accepted October 8, 2011.

doi: 10.1111/j.1537-2995.2011.03450.x

TRANSFUSION 2012;52:298-306.

significant controversy about the relationship of XMRV and/or MLVs to human disease. Furthermore, there is increasing evidence that XMRV is a laboratory artifact resulting from recombination of two endogenous murine retroviral proviruses during passage of prostate tumor cells in mice.[8] Several additional studies have suggested that the findings of the two positive studies reporting a linkage between XMRV and/or MLV and CFS are attributable to contamination via one or more routes (e.g., reagents, samples, techniques) rather than to human infection.[5,6] A recent multicenter study involving nine laboratories testing replicate samples from 15 patients or individuals with a history of XMRV positivity and 15 control subjects has shown that currently used tests, including those from two groups that were part of the study by Lombardi and colleagues,[2] do not reliably detect markers of XMRV. While all but two assays correctly identified blinded negative and positive control samples, no assay could detect XMRV in patients previously characterized as XMRV-infected.[9] However, it has become apparent that XMRV is a bona fide virus with the capability of readily infecting human cells, at least in vitro,[8] although recent data suggest that the virus itself may not be effective in infecting humans in vivo.[10] It has been shown that XMRV can elicit a weak, transient virus-specific immune response in experimentally infected rhesus macaques.[11] Nevertheless, unexpectedly high reported prevalence rates for XMRV and/or MLV markers in healthy human controls warrant further evaluation.

In the context of blood safety, there is concern about the impact of retroviruses, irrespective of their relationship to disease. More specifically, the potential for mutation and acquisition of new pathogenic properties (particularly in the context of a species jump) must always be considered. With respect to XMRV, the NIH has sponsored two groups to examine the risk of this virus to blood safety and its relationship to CFS; each of the two study groups involves multiple investigators with considerable expertise and prior publications in this arena. The first, sponsored by the National Heart, Lung, and Blood Institute, included the examination of patients who were previously found positive in the two referenced studies, described above,[2,4] and the second, an NIH-sponsored study group that focused on patients with well-defined CFS; the second study has not yet been completed. Reports of the activities of the NHLBI group, referred to as the Scientific Research Working Group (SRWG) have been published.[9,12] In addition, the AABB has also provided information and advice to their membership regarding management of presenting blood donors with a history of CFS.[13]

The objective of this study was to determine the prevalence of XMRV infection in US blood donors and to examine the risk of transfusion transmission. High-throughput microparticle-based chemiluminescence immunoassays (CMIAs)[14,15] and transcription-mediated amplification (TMA)[16] were used to evaluate the prevalence of XMRV and/or MLV antibodies and XMRV RNA among routine blood donors and in a population of highly transfused patients and their donors. For the transfusion transmission study, we used a previously characterized donor-recipient repository maintained by the American Red Cross (ARC) and Yale University.[17,18]

MATERIALS AND METHODS

Samples

Four groups of blood donor samples were obtained for XMRV RNA or XMRV and/or MLV antibody testing. First, plasma containing ethylenediaminetetraacetate (EDTA) as an anticoagulant from 1435 unlinked surplus blood donation samples collected in the Charlotte, North Carolina area by the ARC in early 2010 were submitted to Gen-Probe, Inc. (San Diego, CA) for an initial evaluation of the specificity of their assay. Submitted samples tested nonreactive in all routine blood donor screening tests and were frozen within 72 hours of collection. In addition, as part of the specificity evaluation, 97 human T-lymphotropic virus (HTLV)-1 and/or -2 antibody confirmed-positive, ACD-plasma samples identified through routine blood donation screening at the ARC from calendar years 2008 through 2010 were submitted for testing. HTLV confirmed-positive samples including those containing antibodies to HTLV-1 (45), HTLV-2 (30), or both HTLV-1 and -2 (22) were frozen within 24 hours of collection. The HTLV confirmed-positive samples were obtained directly from the retained frozen plasma component of the donated blood unit.

The prevalence study samples included a total of 13,399 unlinked paired serum and EDTA-plasma samples obtained from surplus blood donation samples found nonreactive in all routine blood donor screening tests; samples were frozen within 72 hours of collection. The samples were collected from six ARC blood center collection areas (Atlanta, GA; Baltimore, MD; Boston, MA; Detroit, MI; Los Angeles, CA; and Portland, OR) during June to September 2010. Each location contributed between 2000 and 2600 samples.

Finally, 3741 serum samples were obtained that represented the available retention samples from all blood units transfused to a population of frequently transfused recipient-patients, as previously described.[17,18] For the recipients of this latter group of blood components, there were a total of 830 evaluable blood samples (CPD or EDTA-plasma) representing pre- and sequential post-transfusion samples from 109 of the patients in the same study.[17,18] Recipients in this study only received blood from the 3741 donors, except on rare occasions where other components were transfused.

TABLE 1. XMRV and/or MLV antibody reactivity to individual recombinant antigens by metropolitan area for six US regions*

Region*	Number tested	Number p15E reactive	Percent†	Number gp70 reactive	Percent†	Number p30 reactive
Atlanta, GA	2,385	6	0.25	0		1
		0		19	0.80	1
Boston, MA	2,631	4	0.15	0		0
		0		21	0.80	0
Los Angeles, CA	2,142	3	0.14	0		0
		0		19	0.89	0
Detroit, MI	2,020	8	0.40	0		0
		0		9	0.45	0
Portland, OR	2,008	5	0.25	0		0
		0		12	0.60	0
Baltimore, MD	2,213	3	0.14	0		0
		0		13	0.59	0
Total	13,399	29	0.22	93	0.69	

* Chi-square for p15E (5 d.f.) = 5.06, p = 0.42. Chi-square for gp70 (5 d.f.) = 4.38, p = 0.50.
† The differences in regional prevalence rates are not statistically significant.

Antibody testing

Samples were separately tested for antibodies to XMRV and/or MLV p15E (transmembrane) and gp70 (surface envelope) antigens using prototype CMIAs, processed on an automated analyzer (ARCHITECT, Abbott Diagnostics, Abbott Park, IL).[9,10,14,15] These assays have been shown to be both specific (99.5%-99.9%) and highly sensitive (100%), on the basis of studies performed in rhesus macaques and blood donors.[14] Any sample that gave a signal equal to or greater than the cutoff value (sample-to-cutoff ratio [S/CO] $\geq$ 1.00) was repeated in duplicate and those samples with repeatedly reactive results for either marker were also tested for antibodies to the XMRV and/or MLV p30 antigen (capsid), using the same test method. Repeat reactivity (S/CO $\geq$ 1.0) to all three antigens is required to confirm a positive antibody finding.

RNA testing

Samples were tested for XMRV RNA sequences using a research TMA assay[9,16] and processed on an automated analyzer (TIGRIS System, Novartis Diagnostics, Emeryville, CA); initial reactive samples would have been retested in duplicate had they occurred. This assay has been shown to be more sensitive than other assays when used to test panels of dilutions of the VP62 isolate of XMRV.[12] Analytic analysis using the VP62 isolate demonstrates a sensitivity of 2.5 copies/mL at 95% confidence (95% confidence interval [CI], 1.8-4.8).[12,16]

Statistical analysis

Frequencies of positive findings were calculated including 95% CIs by the mid P exact method (OpenEpi, http://www.openepi.com/OE2.3/Menu/OpenEpiMenu.htm); chi-square analysis was used to estimate regional differences where p values of less than 0.05 were considered significantly different.

TABLE 2. p15E antibody–reactive blood donor sample results from six US regions*

Number	p15E	gp70	p30	RNA
1	1.04	0.08	0.25	0.00
2	2.27	0.09	0.18	0.00
3	9.01	0.10	0.17	0.00
4	1.81	0.10	**7.40†**	0.00
5	1.57	0.12	0.21	0.00
6	1.94	0.12	0.18	0.00
7	1.18	0.09	0.23	0.00
8	1.19	0.14	0.15	0.00
9	13.06	0.10	0.18	0.00
10	5.96	0.09	0.78	0.00
11	5.50	0.10	0.16	0.00
12	2.00	0.13	0.19	0.00
13	1.84	0.11	0.15	0.00
14	6.22	0.11	0.16	0.00
15	3.12	0.08	0.15	0.00
16	1.61	0.27	0.18	0.00
17	3.50	0.09	0.14	0.00
18	1.21	0.07	0.23	0.00
19	1.09	0.09	0.20	0.00
20	3.79	0.10	0.18	0.03
21	1.26	0.13	0.14	0.00
22	7.33	0.09	0.17	0.00
23	1.15	0.10	0.17	0.00
24	2.68	0.05	0.21	0.00
25	2.25	0.09	0.15	0.00
26	3.20	0.06	0.20	0.00
27	1.70	0.05	0.14	0.00
28	1.63	0.06	0.24	0.00
29	1.17	0.06	0.33	0.00

* Reactive results are expressed as means of duplicate retests; n = 29 of 13,399 tested.
† Bolded value is greater than or equal to 1.0 and represents reactive results.

Human subjects review

All studies were approved by the ARC Institutional Review Board; the donor-repository study was also approved by the Yale University Institutional Review Board.

RESULTS

RNA testing

A total of 1435 blood donor samples, nonreactive on all routine screening for blood-borne infections, were tested for XMRV RNA using TMA; none was found to be reactive, with a mean S/CO of 0.18. An S/CO of 1.0 or greater is regarded as reactive. Additionally, none of 97 donor samples confirmed positive for HTLV antibodies was found to be reactive for XMRV RNA (S/CO mean of 0.04). Thus, specificity of the XMRV TMA assay for the 1532 evaluated blood donor samples was 100% (95% CI, 99.8%-100%; n = 1532).

Antibody testing

CMIA was used to identify individual antibodies to recombinant XMRV and/or MLV p15E (transmembrane), gp70 (surface envelope), and p30 (capsid) antigens. Among 13,399 blood donor samples collected from each of six US regions and tested for antibodies to XMRV and/or MLV by CMIA, 29 (0.22%) had isolated reactive results for antibody to p15E, whereas 93 (0.69%) had isolated reactive results for antibody to gp70; no sample had reactivity to both antigens. Isolated antibody reactivity ranged by US region from 0.14% to 0.40% for p15E and 0.45% to 0.89% for gp70 (Table 1): there was no significant difference in prevalence between regions. The respective ranges of S/CO values (mean of duplicate retest determinations) were 1.04 to 13.06 and 1.02 to 32.13. Two samples, one of which was reactive for antibody to p15E and the other for gp70, were found reactive for antibody to p30, with S/CO values of 7.40 and 1.66, respectively (Tables 2 and 3). The distribution of S/CO values for p15E and gp70 antibody reactivity is illustrated in Fig. 1 using a natural log scale to better illustrate samples with weak reactivity. No donor sample had all three XMRV antibody markers. The 122 (29 p15E plus 93 gp70) isolated antibody-reactive samples were also tested for XMRV RNA and were found nonreactive, with S/CO values ranging from 0.00 to 0.14 (Tables 2 and 3). Therefore, there was no evidence of XMRV and/or MLV infection in this population. The prevalence of XMRV and/or MLV antibodies in contemporary US blood donors from six US regions was 0% (95% CI, 0%-0.023%) with no indication of ongoing infection as demonstrated by the lack of viral RNA.

Repository samples

Donors

Among the 3741 donor samples from an established donor-recipient repository,[17,18] five (0.13%) had antibody

TABLE 3. gp70 antibody–reactive blood donor sample results from six US regions*

Number	p15E	gp70	p30	RNA	No.	p15E	gp70	p30	RNA	No.	p15E	gp70	p30	RNA
1	0.30	**1.74**	0.17	0.00	32	0.11	**1.34**	0.16	0.00	63	0.14	**6.10**	0.16	0.00
2	0.17	**3.64**	0.20	0.00	33	0.16	**3.03**	0.26	0.00	64	0.14	**1.29**	0.16	0.00
3	0.15	**2.06**	0.45	0.00	34	0.12	**3.16**	0.47	0.00	65	0.13	**6.11**	0.15	0.00
4	0.13	**4.40**	0.19	0.00	35	0.15	**1.93**	0.16	0.00	66	0.15	**2.51**	0.48	0.00
5	0.13	**3.08**	0.17	0.00	36	0.11	**2.29**	0.15	0.00	67	0.12	**12.53**	0.15	0.00
6	0.13	**1.13**	0.15	0.00	37	0.12	**3.24**	0.16	0.00	68	0.13	**1.31**	0.20	0.00
7	0.15	**5.94**	**1.66**	0.00	38	0.12	**1.14**	0.15	0.00	69	0.13	**5.35**	0.19	0.00
8	0.23	**11.87**	0.14	0.00	39	0.12	**1.86**	0.16	0.00	70	0.13	**23.65**	0.16	0.00
9	0.14	**4.86**	0.19	0.00	40	0.12	**1.02**	0.15	0.00	71	0.14	**6.86**	0.14	0.00
10	0.15	**1.59**	0.16	0.00	41	0.13	**1.45**	0.17	0.00	72	0.12	**1.02**	0.20	0.00
11	0.11	**3.95**	0.20	0.03	42	0.14	**30.30**	0.17	0.00	73	0.10	**5.29**	0.16	0.00
12	0.13	**1.80**	0.18	0.00	43	0.13	**1.57**	0.17	0.00	74	0.13	**1.72**	0.21	0.00
13	0.10	**7.70**	0.17	0.00	44	0.15	**1.53**	0.26	0.00	75	0.14	**1.16**	0.18	0.14
14	0.13	**2.84**	0.17	0.00	45	0.14	**1.57**	0.15	0.00	76	0.28	**3.82**	0.15	0.00
15	0.12	**1.37**	0.20	0.00	46	0.11	**8.83**	0.18	0.00	77	0.11	**3.96**	0.17	0.00
16	0.13	**3.02**	0.19	0.00	47	0.09	**10.66**	0.17	0.00	78	0.13	**8.23**	0.27	0.00
17	0.11	**3.70**	0.18	0.00	48	0.10	**3.16**	0.21	0.00	79	0.14	**1.72**	0.20	0.00
18	0.10	**1.20**	0.26	0.00	49	0.17	**4.37**	0.91	0.12	80	0.16	**1.38**	0.18	0.00
19	0.13	**1.78**	0.25	0.00	50	0.11	**1.02**	0.15	0.00	81	0.18	**1.33**	0.22	0.00
20	0.10	**1.08**	0.17	0.00	51	0.11	**3.05**	0.16	0.00	82	0.16	**1.08**	0.18	0.00
21	0.11	**5.36**	0.18	0.00	52	0.13	**1.16**	0.15	0.00	83	0.13	**1.12**	0.22	0.00
22	0.13	**4.05**	0.20	0.00	53	0.12	**32.13**	0.16	0.00	84	0.14	**1.75**	0.98	0.00
23	0.09	**4.72**	0.15	0.00	54	0.12	**3.65**	0.32	0.00	85	0.13	**4.45**	0.18	0.00
24	0.11	**1.76**	0.28	0.00	55	0.16	**1.04**	0.16	0.00	86	0.13	**1.71**	0.29	0.00
25	0.11	**12.78**	0.21	0.05	56	0.15	**1.24**	0.16	0.00	87	0.17	**1.32**	0.16	0.00
26	0.12	**8.29**	0.17	0.00	57	0.13	**24.05**	0.29	0.01	88	0.15	**11.14**	0.25	0.00
27	0.09	**7.92**	0.18	0.00	58	0.11	**3.70**	0.15	0.00	89	0.13	**2.28**	0.24	0.00
28	0.12	**3.00**	0.27	0.00	59	0.11	**4.86**	0.15	0.00	90	0.10	**2.31**	0.32	0.00
29	0.12	**18.99**	0.16	0.00	60	0.12	**1.45**	0.14	0.00	91	0.17	**1.30**	0.18	0.00
30	0.12	**2.59**	0.18	0.00	61	0.12	**1.54**	0.15	0.00	92	0.13	**2.63**	0.25	0.00
31	0.16	**1.54**	0.19	0.00	62	0.14	**8.57**	0.17	0.00	93	0.13	**21.78**	0.18	0.00

* Reactive results are expressed as means of duplicate retests; n = 93 of 13,399 tested.
Bolded values are greater than or equal to 1.0 and represent reactive results.

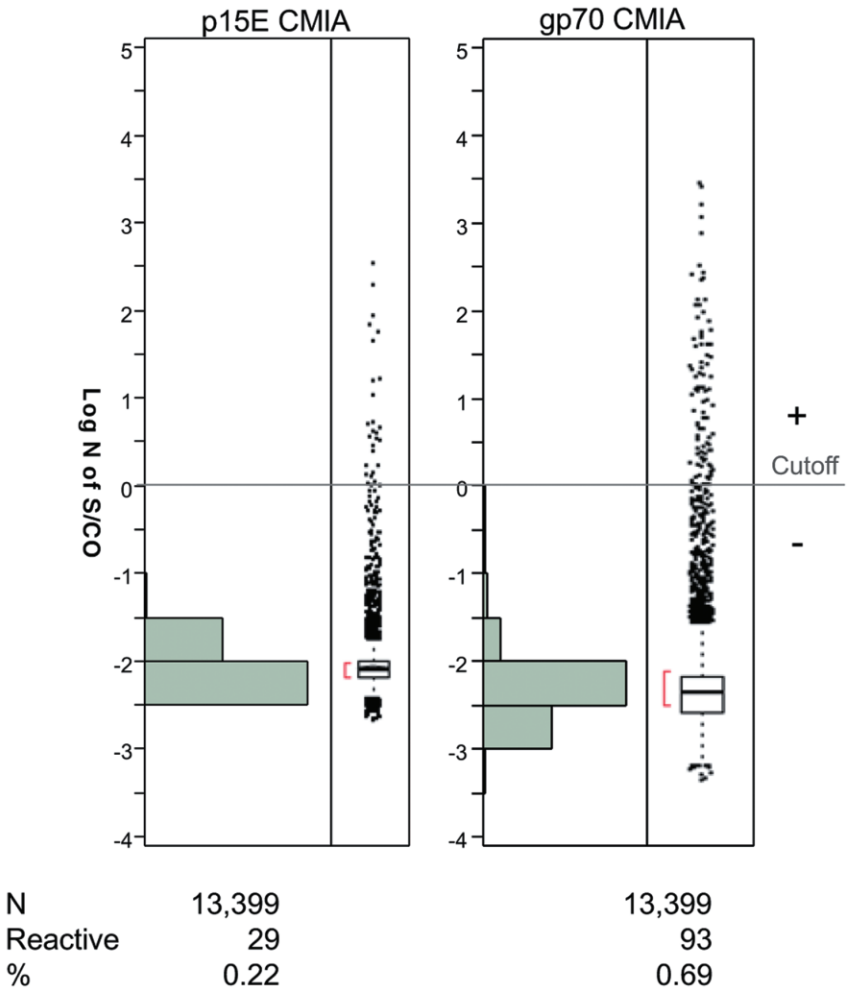

Fig. 1. Signal distributions of p15E CMIA and gp70 CMIA on 13,399 US blood donors from six US regions. The *X* axis shows the sample frequency expressed as number of samples/total population. The *Y* axis represents the CMIA signal expressed in units of natural log-transformed signal ratio of sample to the cutoff (Log N S/CO); values greater than 0 are considered positive. The number (N) of donors tested, number reactive samples, and percentage (%) of reactive samples are summarized beneath each figure.

reactivity to p15E and 20 (0.53%) had antibody reactivity to gp70; no sample had reactivity to both antigens. The distribution of natural log S/CO values is shown in Fig. 2 and a listing of samples with isolated antibody reactivity is shown in Table 4. Among the 25 reactive donor samples, one gp70 antibody-reactive sample was also reactive to p30 (S/CO 4.76 and 4.73, respectively). Thus, no donor sample was confirmed as XMRV and/or MLV antibody positive. The S/CO values for the 20 gp70-reactive samples ranged from 1.04 to 49.11 with the corresponding nonreactive results ranging from 0.11 to 0.17 for p15E and from 0.17 to 0.30 for p30. The S/CO values for the five p15E-reactive samples ranged from 1.09 to 12.47 with corresponding nonreactive results of 0.06 for gp70 and a range of 0.18 to 0.22 for p30. RNA testing was not performed on the donor samples as their storage conditions precluded such testing. These samples represented more than 96% of blood components transfused to the recipient population studied.

Recipients

Among the 830 repository samples representing 109 individual highly transfused recipients, one sample had p15E antibody reactivity and 20 samples, representing two recipients, had reactivity to gp70. No recipient sample had reactivity to both antigens or to p30 (Table 5). Recipient 1 was a 44-year-old male with beta thalassemia: his first sample, taken on September 29, 2004, had a weak isolated antibody signal to gp70 (S/CO, 1.53). Subsequently, he received a total of 73 red blood cell (RBC) and six fresh-frozen plasma units through the period ending May 23, 2006. Among the 21 additional samples tested during the period of transfusion, 18 had weak isolated reactivity to gp70 (S/CO ranged from 1.06-1.64). The remaining three samples were gp70 nonreactive with reactivity just under the assay cutoff (S/CO range, 0.93-0.99); antibodies to p15E and p30 were nonreactive for all samples (S/CO ranges of 0.13-0.16 and 0.19-0.26, respectively). This same recipient had tested immunoglobulin (Ig)G weakly reactive to three of four markers for which the repository samples had previously been tested (parvovirus B19, cytomegalovirus, and *Chlamydia pneumoniae*). Recipient 2 was a 63-year-old male with coronary artery disease; he had weak isolated p15E reactivity (S/CO, 1.64) in a pretransfusion sample with nonreactive results for both gp70 and p30 (S/CO, 0.08 and 0.32, respectively); no follow-up samples were available for further evaluation. Recipient 2 was also IgG weakly reactive for parvovirus B19 and *C. pneumoniae*. Recipient 3 was an 18-year-old female with sickle cell disease. Her initial sample, on April 28, 2005, was nonreactive for p15E and gp70 (S/CO, 0.18 and 0.10, respectively). She received a total of 41 RBC units in the period up to April 19, 2007. Among seven follow-up samples tested, one had weak isolated reactivity to gp70 (S/CO, 1.12) with nonreactive results for p15E and p30 (S/CO, 0.17 and 0.23, respectively); all six subsequent samples were gp70 nonreactive (S/CO range, 0.06-0.08). Recipient 3 was also IgG weakly reactive for parvovirus

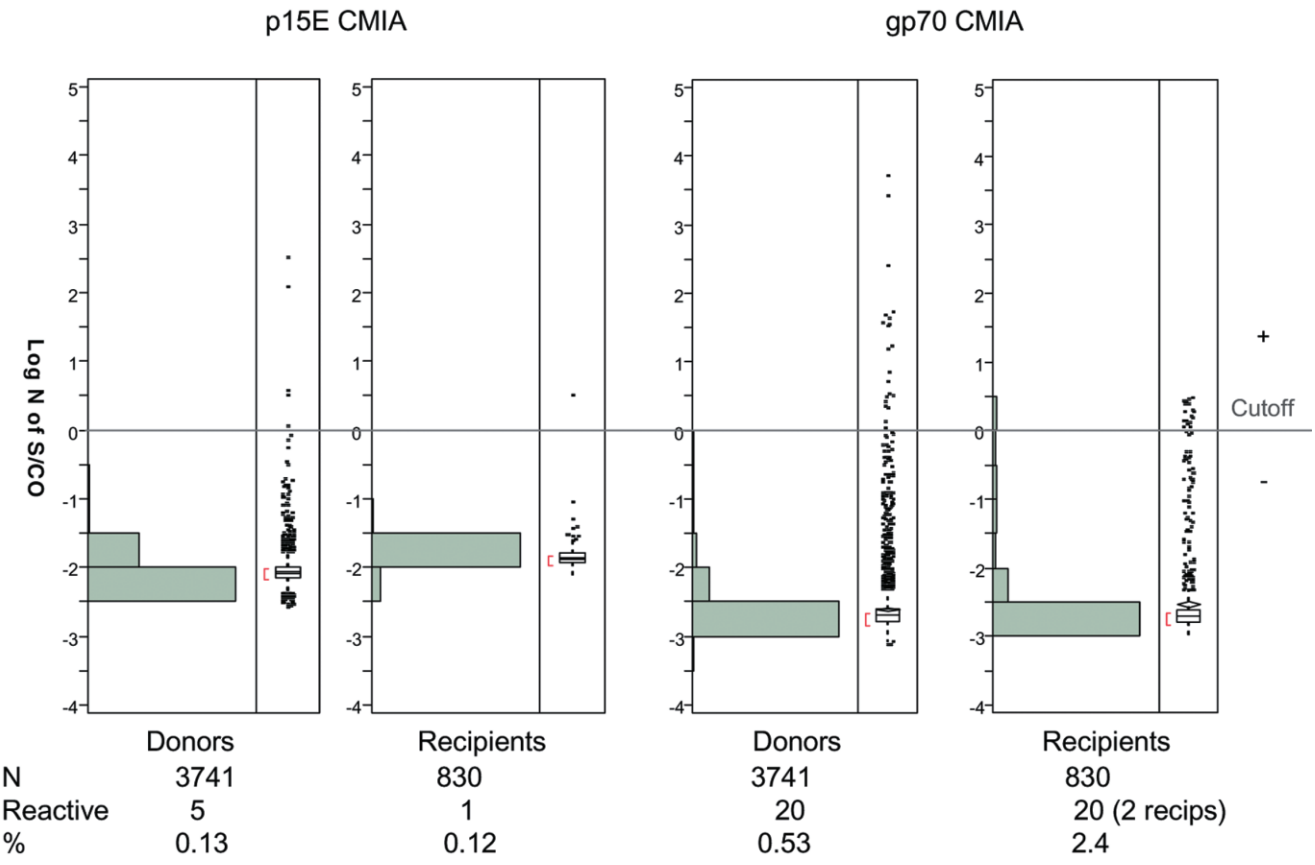

Fig. 2. Signal distributions of p15E CMIA and gp70 CMIA on 3741 US blood donations and 830 recipient samples from a well-characterized donor-recipient repository. The *X* axis shows the sample frequency expressed as number of samples/total population. The *Y* axis represents the CMIA signal expressed in units of natural log-transformed signal ratio of sample to the cutoff (Log N S/CO); values greater than 0 are considered positive. The number (N) of donors and recipients (recips) tested, number of reactive samples, and percentage (%) of reactive samples are summarized beneath each figure.

B19, cytomegalovirus (CMV), *C. pneumoniae*, and *Babesia microti* (*B. microti* unpublished observation). None of the three recipients with isolated antibody reactivity had received blood products from the 25 donors described as having detectable but unconfirmed XMRV and/or MLV antibody reactivity. All 830 recipient samples were nonreactive for XMRV RNA (S/CO, 0.00-0.59). A listing of all observed antibody reactivity and associated RNA-nonreactive results for tested samples from the three described recipients is shown in Table 5.

In summary, 17,249 antibody-tested blood donors and recipients (13,399 + 3741 + 109) and 1763 RNA-tested blood donors and recipients (1435 + 97 + 122 + 109) showed no evidence of XMRV infection, for an antibody prevalence of 0% (95% CI, 0%-0.017%) and RNA prevalence of 0% (95% CI, 0%-0.17%) as shown in Table 6.

DISCUSSION

In this study, we used well-validated, automated tests to evaluate the prevalence of XMRV and/or MLV antibody and XMRV RNA in a large number of blood donors. Using interpretive criteria already established for the antibody tests, we have not found any evidence of confirmed XMRV and/or MLV antibodies among 17,140 (13,399 + 3741) blood donors, implying an upper 95% confidence bound of less than 0.02% prevalence. Furthermore, we have been unable to demonstrate any detectable XMRV RNA in 1435 routine blood donation samples, in any sample with isolated antibody reactivity (122), in donation samples that are positive for HTLV antibodies (97), or from 830 samples collected from 109 highly transfused recipients.

These negative findings among blood donors make the question of transmissibility of XMRV by transfusion somewhat academic. Nevertheless, we have examined an existing donor-recipient repository for evidence of such transmission. We did find a low frequency of antibodies to individual XMRV and/or MLV recombinant antigens among both donors and recipients; however, antibody positivity could not be confirmed by the presence of all three antibody markers and no recipient tested RNA positive. Furthermore, donations with reactive, unconfirmed

TABLE 4. Sample results from antibody-reactive blood donors from the donor-recipient repository*

Donor sample	p15E	gp70	p30
1	0.16	**1.47**	0.30
2	0.17	**5.14**	0.17
3	0.13	**4.74**	4.73
4	0.14	**1.42**	0.19
5	0.13	**11.19**	0.17
6	0.12	**1.76**	0.17
7	0.12	**3.51**	0.22
8	0.13	**5.38**	0.20
9	0.12	**1.83**	0.18
10	0.13	**1.51**	0.18
11	0.12	**1.44**	0.22
12	0.13	**2.20**	0.19
13	0.15	**31.96**	0.19
14	0.12	**6.36**	0.20
15	0.13	**49.11**	0.22
16	0.11	**1.04**	0.23
17	0.11	**4.94**	0.22
18	0.12	**2.18**	0.23
19	0.11	**4.48**	0.18
20	0.12	**3.76**	0.24
1	**2.04**	0.06	0.22
2	**1.09**	0.06	0.18
3	**12.47**	0.06	0.21
4	**1.68**	0.06	0.18
5	**9.30**	0.06	0.20

* Reactive results expressed as means of duplicate retests; n = 25 of 3741 tested.
Bolded values are greater than or equal to 1.0 and represent reactive results.

TABLE 5. Sample results from antibody-reactive recipients from the donor-recipient repository*

Subject	Date of collection	p15E	gp70	p30	RNA
Recipient 1					
1	September 29, 2004	0.15	**1.53**	0.26	0.01
2	October 27, 2004	0.16	**1.54**	0.22	0.05
3	November 17, 2004	0.14	**1.64**	0.20	0.08
4	December 6, 2004	0.15	**1.36**	0.22	0.06
5	January 10, 2005	0.15	**1.34**	0.22	0.05
6	January 26, 2005	0.16	**1.06**	0.21	0.04
7	February 25, 2005	0.14	**1.29**	0.22	0.04
8	March 18, 2005	0.14	**1.49**	0.23	0.05
9	April 25, 2005	0.14	**1.45**	0.20	0.20
10	June 6, 2005	0.14	**1.24**	0.26	0.06
11	June 27, 2005	0.14	**1.28**	0.24	0.41
12	July 18, 2005	0.13	**1.20**	0.24	0.09
13	August 8, 2005	0.14	0.97		0.00
14	August 29, 2005	0.15	**1.10**	0.22	0.13
15	October 10, 2005	0.13	0.99		0.01
16	November 7, 2005	0.13	0.93		0.59
17	November 28, 2005	0.14	**1.19**	0.22	0.00
18	January 11, 2006	0.14	**1.18**	0.24	0.00
19	February 6, 2006	0.13	**1.44**	0.19	0.02
20	March 6, 2006	0.15	**1.16**	0.22	0.04
21	March 27, 2006	0.15	**1.14**	0.20	0.00
22	April 17, 2006	0.15	**1.12**	0.22	0.05
Recipient 2					
1	October 22, 2004	**1.64**	0.08	0.32	0.08
Recipient 3					
1	April 28, 2005	0.18	0.10		0.00
2	November 30, 2005	0.17	**1.12**	0.23	0.10
3	March 2, 2006	0.16	0.06		0.03
4	October 4, 2006	0.21	0.07		0.00
5	November 22, 2006	0.21	0.07		0.05
6	February 20, 2007	0.19	0.08		0.04
7	March 21, 2007	0.27	0.08		0.05
8	April 19, 2007	0.35	0.08		0.03

* Reactive results expressed as means of duplicate tests; n = 21 of 830 tested.
Bolded values are greater than or equal to 1.0 and represent reactive results.

antibody markers were not associated with the three recipients that demonstrated such markers; no isolated antibody-reactive donation was associated with a reactive recipient. Therefore, we conclude that, whatever the origin of the isolated and unconfirmed antibody reactivity that we observed, these were not attributable to transfusion-transmitted XMRV and/or MLV. The presence of isolated IgG antibodies to either or all agents previously investigated in these studied recipients (i.e., parvovirus B19, CMV, *C. pneumoniae*, and *B. microti*) indicates either specific immune responses to these agents (in some case attributable to passive transfer) or just as likely, nonspecific reactivity attributable to the fact that these recipients were highly transfused.

Other studies have reported the absence of reliable detection of markers of XMRV and/or MLV among small numbers of blood donors (236 human immunodeficiency virus–infected blood donors in Africa by polymerase chain reaction [PCR] and 391 routine blood donors in China by PCR and culture),[19,20] but our study is the first to examine a large and geographically diverse population of healthy, routine US blood donors for evidence of active XMRV and/or MLV infection. Although the SRWG group concluded that their study indicated "that routine blood donor screening for XMRV/P-MLV is not warranted at this time," this conclusion was based on the inability of the evaluated tests to reliably detect the presence of XMRV markers among samples drawn from only 15 subjects (14 CFS patients and one relative of a patient), previously found positive for XMRV and/or MLV. There were also 15 healthy controls who were blood donors or laboratory staff.[9] It should be noted that the tests used in our studies were also evaluated in the SRWG studies and are well-validated and are sensitive when used on relevant control preparations.[9-12,14,15] We have shown that, in contrast to the studies of Lombardi and colleagues[2] and Lo and colleagues[3] the measured prevalence of markers of infection among healthy donors is zero and that the upper 95% CI for XMRV-related antibody and RNA is, respectively, 0.017 and 0.17%. Further, direct observation of viral markers among blood donors and the recipients of their blood showed no evidence of any transmission of XMRV-related viruses. We recognize that studies in rhesus macaques showed that markers of XMRV in the blood of inoculated animals were transient and weak,[11] suggesting that these

TABLE 6. Blood donor and recipient studies for XMRV-related antibodies and RNA

Population	Test: number reactive/total tested or reactive subset tested						Number confirmed positive
	Anti-p15E	Anti-gp70	Anti-p30*	Anti-p15E and p30	Anti-gp70 and p30	RNA	
Random blood donors, n = 1,435 (TMA validation)	ND	ND	ND	ND	ND	0	0
HTLV-positive blood donors, n = 97 (TMA validation)	ND	ND	ND	ND	ND	0	0
Random blood donors, n = 13,399	29	93	2/122	1/29	1/93	0/122	0
Repository blood donors, n = 3,741	5	20	1/25	0/5	1/20	ND	0
Repository blood recipients (samples), n = 830†	1	20	0/21	0/1	0/20	0/830	0
Repository blood recipients (patients), n = 109	1	2	0/3	0/1	0/2	0/109	0
Total (%) individuals antibody tested, n = 17,249 (13,399 + 3,741 + 109)	35 (0.20)	115 (0.67)	3/150 (2.00)	1/35 (2.86)	2/115 (1.72)		0
Total individuals RNA tested, n = 1,763 (1,435 + 97 + 122 + 109)	ND	ND	ND	ND	ND	0	0

* Anti-p30 testing was performed on anti-p15E– or anti-gp70–reactive samples.
† Results not part of total.
ND = not done.

markers would be more likely to occur after recent infection. Thus, a limitation of our study is that detection of XMRV markers might be expected to be infrequent. Nevertheless, we feel that it is important to contrast our findings with the high prevalence rates among controls that were previously reported by Lombardi and coworkers[2] and Lo and coworkers.[4]

The lessons learned from the 2-year exercise since the threat of XMRV to blood safety was initially raised[3] warrant mention. The United States and the world monitored the scientific literature closely, mobilized technical, regulatory, and policy groups to identify gaps and investigate immediate actions to protect donor and recipient safety including the ongoing exclusion of donors with a medical diagnosis of CFS. Through extensive efforts, the scientific process was invoked and successful in that the threat of XMRV was given a priority status and fully investigated and through the generation of data using targeted study populations can now be concluded to pose no current threat. The models for investigating emerging infectious diseases that challenge transfusion safety will by necessity differ depending on the specific agent, its epidemiology, and a host of other factors. The findings of our study, coupled with the prior studies that did not confirm any association of XMRV and/or MLV with human disease, indicate that these viruses do not currently pose a threat to blood recipients or to public health.[9,10,12,21] Thus, we conclude that no further action relating to XMRV and/or MLV and blood safety is necessary.

ACKNOWLEDGMENTS

We acknowledge those staff at the American Red Cross National Testing Laboratories who provided the blood donor samples. In addition, Erin Moritz, PhD, of the ARC Holland Laboratory assisted in the preparation of the repository samples for testing. We thank Bailin Tu, PhD, and Bruce Dille, PhD, from Abbott Diagnostics for preparation of recombinant gp70 protein. We also acknowledge Dat Do and Tan Le for their technical assistance at Gen-Probe. We thank the American Red Cross, Abbott Laboratories, and Gen-Probe Inc. for providing financial and operational support for this study. The products reported in this study are not for commercial use.

CONFLICT OF INTEREST

RYD, KD, YW, SZ, DEK, and SLS have no conflicts. JH, XQ, PS, and GS are employees and shareholders of Abbott Laboratories. JML, KG, and JMC are employees of Gen-Probe, Inc.

REFERENCES

1. Urisman A, Molinaro R, Fischer N, Plummer S, Casey G, Klein E, Malathi K, Magi-Galluzzi C, Tubbs R, Ganem D,

Silverman R, DeRisi J. Identification of a novel gammaretrovirus in prostate tumors of patients homozygous for R462Q *RNASEL* variant. PLoS Pathog 2006;2:e25.
2. Lombardi VC, Ruscetti FW, Das Gupta J, Pfost MA, Hagen KS, Peterson DL, Ruscetti SK, Bagni RK, Petrow-Sadowski C, Gold B, Dean M, Silverman RH, Mikovits JA. Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. Science 2009;326:585-9.
3. Coffin JM, Stoye JP. A new virus for old diseases? Science 2009;326:530-1.
4. Lo SC, Pripuzova N, Li B, Komaroff AL, Hung GC, Wang R, Alter HJ. Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors. PNAS 2010;107:15874-9.
5. American Association of Blood Banks. Published studies on XMRV and MLRV findings in human diseases and the general population. 2011. [cited 2011 Nov 1]. Available from: URL: http://www.aabb.org/resources/bct/eid/Documents/xmrvtable.pdf
6. Alberts B. Editorial expression of concern. Science 2011; 333:335.
7. Silverman RH, Das Gupta J, Lombardi VC, Ruscetti FW, Pfost MA, Hagen KS, Peterson DL, Ruscetti SK, Bagni RK, Petrow-Sadowski C, Gold B, Dean M, Mikovits JA. Partial retraction. Science 2011. Published online 22 Sept 2011. doi: 10.1126/science1212182.
8. Paprotka T, Delviks-Frankenberry KA, Cingöz O, Martines A, Kung HJ, Tepper CG, Hu WS, Fivash MJ, Coffin JM, Pathak VK. Recombinant origin of the retrovirus XMRV. Science 2011;333:97-101.
9. Simmons G, Glynn SA, Komaroff AL, Mikovits JA, Tobler LH, Hackett J Jr, Tang N, Switzer WM, Heneine W, Hewlett IK, Zhao J, Lo SC, Alter HJ, Linnen JM, Gau K, Coffin JM, Kearney MF, Ruscetti FW, Pfost MA, Bethel J, Kleinman S, Homberg JA, Busch MP; Blood Scientific Research Working Group (SRWG). Failure to confirm XMRV/MLVs in the blood of patients with chronic fatigue syndrome: a multi-laboratory study. Science 2011. Published online 22 September 2011. doi: 10.1126/science1213841.
10. Knox K, Carrigan D, Simmons G, Teque F, Zhou V, Hackett J Jr, Qiu X, Luk KC, Schocheteman G, Knos A, Kogelnik AM, Levy JA. No evidence of murine-like gammaretroviruses in CFS patients previously identified as XMRV-infected. Science 2011;333:94-7.
11. Onlamoon N, Das Gupta J, Sharma P, Rogers K, Suppiah S, Rhea J, Molinaro RJ, Gaughan C, Dong B, Klein EA, Qiu X, Devare S, Schochetman G, Hackett J Jr, Silverman RH, Villinger F. Infection, viral dissemination and antibody responses of Rhesus macaques exposed to the human gammaretrovirus XMRV. J Virol 2011;85:4547-57.
12. Simmons G, Glynn SA, Holmberg JA, Coffin JM, Hewlett IK, Lo SC, Mikovits JA, Switzer WM, Linnen JM, Busch MP; the Blood XMRV Scientific Research Working Group. The Blood Xenotropic Murine Leukemia Virus-Related Virus Scientific Research Working Group: mission, progress, and plans. Transfusion 2011;51:643-53.
13. Klein HG, Dodd RY, Hollinger FB, Katz LM, Kleinman S, McCleary KK, Silverman RH, Stramer SL; the AABB Interorganizational Task Force on XMRV. Xenotropic murine leukemia virus-related virus (XMRV) and blood transfusion: report of the AABB interorganizational XMRV task force. Transfusion 2011;51:654-61.
14. Qiu X, Swanson P, Luk KC, Tu B, Villinger F, Das Gupta J, Silverman RH, Klein EA, Devare S, Schochetman G, Hackett J Jr. Characterization of antibodies elicited by XMRV infection and development of immunoassays useful for epidemiologic studies. Retrovirology 2011;7:68-84.
15. Oakes B, Qiu X, Levine S, Hackett J Jr, Huber BT. Failure to detect XMRV-specific antibodies in the plasma of CFS patients using highly sensitive chemiluminescence immunoassays. Adv Virol 2011. doi: 10.1155/2011/854540.
16. Linnen J. Reported at US FDA Blood Products Advisory Committee Meeting. December 14, 2010. [cited 2011 Nov 1]. Transcript available from: URL: http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/BloodProductsAdvisoryCommittee/ucm239304.htm
17. Zou S, Wu Y, Cable R, Dorsey K, Tang Y, Hapip CA, Melmed R, Trouern-Trend J, Carrano D, Champion M, Fujii K, Fang C, Dodd RY. A prospective study of multiple donor exposure blood recipients: surveillance value and limitations for hemovigilance. Transfusion 2010;50:128-38.
18. Wu Y, Zou S, Cable R, Dorsey K, Tang Y, Hapip CA, Melmed R, Trouern-Trend J, Wang JH, Champion M, Fang C, Dodd RY. Direct assessment of cytomegalovirus transfusion-transmitted risks after universal leukoreduction. Transfusion 2010;50:776-86.
19. Tang S, Zhao J, Viswanath VR, Nyambi PN, Redd AD, Dastyar A, Spacek LA, Quinn TC, Wang X, Wood O, Gaddam D, Devadas K, Hewlett IK. Absence of detectable XMRV in plasma or PBMC of human immunodeficiency virus type-1 infected blood donors and individuals in Africa. Transfusion 2011;51:463-8.
20. Mi Z, Lu Y, Zhang S, An X, Wang X, Chen B, Wang Q, Tong Y. Absence of xenotropic murine leukemia virus-related virus in blood donors in China. Transfusion 2012;52:326-31.
21. Shin CH, Bateman L, Schlaberg R, Bunker AM, Leonard CJ, Hughen RW, Light AR, Light KC, Singh IR. Absence of XMRV retrovirus and other related viruses in patients with chronic fatigue syndrome. J Virol 2011;85:7195-202.