資料３－１

供血者からの遡及調査の進捗状況について
（目次）

事　務　連　絡
平成２４年１月２４日

日本赤十字社血液事業本部　御中

薬事・食品衛生審議会血液事業部会事務局
厚生労働省医薬食品局血液対策課

供血者からの遡及調査の進捗状況について

標記につきましては、平成２３年１１月１８日付け血安第４１９号にて貴社血液事業本部長より資料の提出があり、これを平成２３年度第３回血液事業部会運営委員会に提出したところです。今般、平成２４年３月１４日（水）に平成２３年度第４回血液事業部会運営委員会が開催されることとなりましたので、下記の事項について改めて資料を作成いただき、平成２４年２月７日（火）までに当事務局あて御提出いただきますようお願いいたします。

記

1．「供血者の供血歴の確認等の徹底について」（平成１５年６月１２日付け医薬血発第0612001号）に基づく遡及調査に係る以下の事項
（１）遡及調査実施内容
①　調査の対象とした献血件数
②　上記①のうち、調査の対象とした輸血用血液製剤の本数
③　上記②のうち、医療機関に情報提供を行った本数
（２）個別NAT関連情報
①　（１）①のうち、個別NATの結果が陽性となった献血件数
②　上記①のうち、医療機関へ供給された製剤に関する報告件数
③　上記②のうち、受血者情報が判明した件数
④　上記③のうち、医薬品副作用感染症報告を行った件数

2．資料の作成に当たっての留意事項
①　本数又は件数については、病原体別及びその合計を明らかにすること。また、上記（１）の③及び（２）の①～③については、対象期間ごとに本数又は件数を記載すること。
②　本数又は件数については、平成２３年１１月１８日付け血安第４１９号の提出時において判明したものに、その後の遡及調査の進展状況を反映させて記載すること。

血安第４１号

平成２４年２月７日

厚生労働省医薬食品局血液対策課長 様

日本赤十字社

血液事業本部長

供血者からの遡及調査の進捗状況について（回答）

平成 24 年 1 月 24 日付事務連絡によりご連絡のありました標記の件について、別紙により報告いたします。

別紙

供血者から始まる遡及調査実施状況

平成23年11月30日現在

対象期間	平成21年4月1日 ～ 平成22年3月31日			平成22年4月1日 ～ 平成23年3月31日			平成23年4月1日 ～ 平成23年11月30日		
	HBV	HCV	HIV	HBV	HCV	HIV	HBV	HCV	HIV
(1)遡及調査実施内容									
① 調査の対象とした献血件数(個別NAT実施件数)									
1)総数	1,806			1,852			1,640		
2)個別件数	1,688	69	49	1,730	74	48	1,588	34	18
② 上記①のうち、調査の対象とした輸血用血液製剤の本数									
1)総数	2,014			2,072			1,820		
2)個別本数	1,877	84	53	1,934	82	56	1,760	42	18
③ 上記②のうち、医療機関に情報提供を行った本数									
1)総数	2,014			2,072			1,634		
2)個別本数	1,877	84	53	1,934	82	56	1,580	36	18
(2)個別NAT関連情報									
① 遡及調査実施対象〔(1)①〕のうち、個別NATの結果が陽性となった献血件数									
1)総数	144			100			75		
2)個別件数	144	0	0	100	0	0	75	0	0
② 上記①のうち、医療機関へ供給された製剤に関する報告件数									
1)使用された本数	140	0	0	98	0	0	77	0	0
2)医療機関調査中	0	0	0	0	0	0	0	0	0
3)院内で廃棄	6	0	0	5	0	0	1	0	0
4)不明	6	0	0	3	0	0	0	0	0
計	152	0	0	106	0	0	78	0	0
③ 上記②のうち、受血者情報が判明した件数									
1)陽転事例	1	0	0	4	0	0	6	0	0
2)非陽転事例	55	0	0	29	0	0	29	0	0
3)死亡	55	0	0	44	0	0	32	0	0
4)退院・未検査	19	0	0	15	0	0	9	0	0
5)陽性だが輸血前不明	10	0	0	6	0	0	1	0	0
計	140	0	0	98	0	0	77	0	0
④ 上記③のうち、医薬品副作用感染症報告を行った件数									
報告件数	1	0	0	4	0	0	4	0	0

*血液製剤等に係る遡及調査ガイドライン(平成20年12月26日一部改正)に基づく遡及調査対応基準を適用。

HBV ：HBs抗原CLEIA法確認試験（中和試験）又は個別NAT陽性の場合は遡及調査を行う。

：HBc抗体CLEIA法陽転の場合は遡及調査を行う。

HCV ：HCV抗体CLEIA法陽転の血液及び前回の血液について個別NATを実施し、いずれかが陽性の場合は遡及調査を行う。

HIV ：HIV抗体CLEIA法で陽転し、確認試験（WB法）又は個別NAT陽性の場合は遡及調査を行う。

共通 ：スクリーニングNAT陽転の場合は遡及調査を行う。

〈参考〉

供血者から始まる遡及調査実施状況

対象期間	平成11年4月1日～平成18年3月31日			平成18年4月1日～平成19年3月31日			平成19年4月1日～平成20年3月31日			平成20年4月1日～平成21年3月31日		
	HBV	HCV	HIV	HBV	HCV	HIV	HBV	HCV	HIV	HBV	HCV	HIV
① 調査の対象とした献血件数												
1）遡及調査の対象件数	23,104			2,193			2,694			5,219		
② 上記①のうち、個別NAT検査を実施した本数（検体数）												
1）本数（検体数）	23,104			2,193			2,694			5,219		
2）実施率	100%			100%			100%			100%		
③ 上記②のうち陽性が判明した本数												
本数	311	3	1	60	1	0	25	0	0	118	0	0
④ 上記①のうち医療機関に情報提供を行った件数												
1）血液製剤数（総数）	33,114			2,408			2,867			4,034		
個別本数				2,062	288	58	2,444	345	78	3,552	417	65
2）情報提供数	33,114			2,408			2,708			3,469		
個別件数				2,062	288	58	2,319	317	72	3,150	254	65
＊平成11年4月1日～平成17年3月31日までの情報提供数には、医療機関の廃院等による追跡不能数930件を含む												
⑤ 上記③のうち医療機関へ供給された製剤に関する報告件数												
1）使用された本数	326	3	1	51	2	0	26	0	0	94	0	0
2）医療機関調査中	0	0	0	0	0	0	0	0	0	0	0	0
3）院内で廃棄	16	0	0	2	0	0	2	0	0	5	0	0
4）不明	7	1	0	0	0	0	0	0	0	0	0	0
計	349	4	1	53	2	0	28	0	0	99	0	0
⑥ 上記⑤のうち、受血者情報が判明した件数												
1）陽転事例	17	1	1	4	1	0	4	0	0	3	0	0
2）非陽転事例	69	0	0	11	0	0	9	0	0	30	0	0
3）死亡	118	2	0	31	1	0	10	0	0	42	0	0
4）退院・未検査	15	0	0	0	0	0	0	0	0	0	0	0
5）陽性だが輸血前不明	7	0	0	1	0	0	0	0	0	0	0	0
計	226	3	1	47	2	0	23	0	0	75	0	0
＊個別NAT陰性（NATウインドウピリオド）の遡及調査対象血液の輸血により、受血者が陽転した例を含む												
⑦ 上記⑥のうち、医薬品副作用感染症報告を行った件数												
報告件数	16＊	1	1	5	1	0	4	0	0	3	0	0
ウイルス別合計	HBV:28 HCV:2 HIV:1											

＊受血者情報の陽転事例のうち医薬品感染症報告が行われていない平成12年3月の事例は、献血血液が遡及調査の対象（個別HBV-NAT陽性）となり、受血者の陽転化情報が得られたが、患者は原疾患により死亡した事例である。

＊平成20年度は、遡及調査対応基準を改定した。（同年10月29日開催「薬事・食品衛生審議会血液事業部会運営委員会」にて了承済）

薬事法第７７条の４の３に基づく回収報告状況

○平成２３年１１月～平成２４年１月

報告日	回収開始年月日	回収対象製品	製造番号	対象本数
平成23年11月2日	平成23年11月1日	新鮮凍結血漿-LR「日赤」成分採血由来	54-1231-1591	1
平成23年11月15日	平成23年11月11日	照射赤血球濃厚液-LR「日赤」400mL由来	11-1320-7204	1
平成23年11月22日	平成23年11月17日	照射赤血球濃厚液-LR「日赤」400mL由来	51-1128-2556	1
平成23年12月1日	平成23年11月30日	照射赤血球濃厚液-LR「日赤」400mL由来	31-1325-5652	1
平成23年12月5日	平成23年12月1日	新鮮凍結血漿-LR「日赤」400mL由来	78-8729-9802	1
平成23年12月7日	平成23年12月5日	照射赤血球濃厚液-LR「日赤」400mL由来	24-0129-6075	1
平成23年12月7日	平成23年12月6日	新鮮凍結血漿-LR「日赤」400mL由来	55-1029-1766	1
平成23年12月20日	平成23年12月19日	新鮮凍結血漿-LR「日赤」400mL由来	53-3225-1965	1
平成24年1月11日	平成24年1月11日	照射赤血球濃厚液-LR「日赤」400mL由来	62-2420-5765	1
平成24年1月13日	平成24年1月12日	照射赤血球濃厚液-LR「日赤」400mL由来	50-0822-3961	1
平成24年1月30日	平成24年1月27日	照射赤血球濃厚液-LR「日赤」400mL由来	32-2327-7276	1

資料３－２

血液製剤に関する医療機関からの感染症報告事例等について

輸血用血液製剤で感染が疑われる事例について

（平成２４年２月７日時点。過去５年間分）

【ＨＢＶ感染が疑われた事例】

報告日	輸血された血液製剤	供血者数	供血者検査結果等	同一血液由来の他製剤等について	新規報告
H19. 2. 20	赤血球濃厚液	3 人	保管検体個別 NAT 全て陰性 3 人中 2 人来訪 HBV 関連検査陰性：2 人	原料血漿：3 本全て確保。	平成 19 年 10 月 19 日以降、残る 1 人の来訪なし。
H21. 11. 20	新鮮凍結血漿 血小板製剤 赤血球製剤	45 人	保管検体個別 NAT 全て陰性 感染が疑われる輸血時の製剤の供血者 23 人 23 人中 21 人来訪 HBV 関連検査陰性：21 人	原料血漿：20 本中 2 本確保。18 本使用済み。 新鮮凍結血漿：3 本全て供給済み。 赤血球製剤：22 本全て供給済み。	平成 23 年 6 月 27 日以降、残る 2 人の来訪なし。

平成23年度感染症報告事例のまとめについて

（平成23年11月～平成23月12月報告分）

1　平成23年11月から23年12月までに報告（新規及び追加）があった感染症報告（疑い事例を含む。供血者からの情報により開始した遡及調査によるものを除く）は、輸血用血液製剤17件である。

輸血用血液製剤の内訳は、

（１）HBV感染報告事例：	8件
（２）HCV感染報告事例：	7件
（３）HIV感染報告事例：	0件
（４）その他の感染症報告例：	2件

2　HBV感染報告事例

（１）輸血前後に感染症検査でHBV-DNA、HBs抗原等が陽転した事例は8件。輸血後NATで陰性又は輸血前後で陽性は0件。

（２）使用された輸血用血液製剤を提供した献血者の保管検体の個別NAT陽性の事例は4件。

（３）劇症化又は輸血後に死亡（原疾患又は他の原因による死亡を除く）したとの報告を受けた事例は0件。

3　HCV感染報告事例

（１）輸血前後に抗体検査（又はHCV-RNA）等が陽転した事例は7件。輸血後NATで陰性又は輸血前後で陽性は0件。

（２）使用された輸血用血液製剤を提供した献血者の保管検体の個別NAT陽性事例は0件。

（３）劇症化又は輸血後に死亡（原疾患又は他の原因による死亡を除く）したとの報告を受けた事例は0件。

4　HIV感染報告事例

（１）輸血前後に抗体検査等が陽転した事例は0件。

（２）使用された輸血用血液製剤を提供した献血者の保管検体の個別NAT陽性事例は0件。

（３）輸血後に死亡（原疾患又は他の原因による死亡を除く）したとの報告を受けた事例は0件。

5　その他の感染症報告事例

（１）B型肝炎及びC型肝炎以外の肝障害報告事例は0件。

（２）細菌等感染報告事例において、使用された輸血用血液製剤を提供した献血者の保管検体の無菌試験陽性事例は0件。

（３）輸血後に死亡（原疾患又は他の原因による死亡を除く）したとの報告を受けた事例は0件.

日赤番号	識別番号	FAX受付日	報告受領日	販売名(一般名)	患者性別	年代	原疾患	感染症名	投与年月	投与前検査(年月)	投与後検査(年月)	日赤投与前検査	日赤投与後検査	受血者個別NAT	献血者個別NAT	併用血液製剤等	備考	使用単位数	供血者再献血※	同一供血者製剤確保※	同一供血者製剤使用※	感染症等転帰	転帰	供血者発遡及の場合の供血者保管検体	供血者発遡及の場合の供血者の検査値
				輸血によるHBV感染報告例(疑い例を含む。)																					
供血者陽性事例																									
3-1100077	A-11000066	2011/10/20	2011/11/1	照射濃厚血小板-LR(人血小板濃厚液(放射線照射))	女	60	血液疾患	B型肝炎	11/06	HBsAg(−) HBsAb(−) HBcAb(−) (11/05)	HBV-DNA(−) HBsAg(−) HBsAb(+) HBcAb(−) (11/06)(遡及調査による情報提供により実施した検査結果) HBV-DNA(+) HBsAg(−) HBsAb(+) HBcAb(−) (11/09)(遡及調査による情報提供により実施した検査結果)	-	HBV-DNA(−) HBsAg(−) HBsAb(+) HBcAb(−) (11/06) HBV-DNA(+) HBsAg(−) HBsAb(+) HBcAb(−) (11/09)	陽性(輸血後)	保管検体1本についてHBV-DNA(+)		【献血者陽転化情報】 当該献血 2011年5月30日 HBV関連検査 陰性 個別HBV-NAT 陽性(遡及調査) 次回献血 2011年6月14日 スクリーニングNAT(HBV) 陽性(陽転献血) 担当医より「輸血血液と感染症の因果関係はあると考える」とのコメントが得られた。	15単位	-	1本の原料血漿を製造、確保済み。	-	非重篤	未回復		患者検体と献血者検体とでPreS/S領域を含むP領域の前半部の1550bpの塩基配列を比較したところ、すべて一致した。両者のHBV-DNAはGenotypeBで塩基配列からSubtypeはadwと推定された。
3-1100078	A-11000068	2011/10/28	2011/11/10	新鮮凍結血漿-LR(新鮮凍結人血漿)	男	70	循環器疾患	B型肝炎	09/09	HBsAg(−)(09/08)	HBsAg(−)、HBcAb(+)、IgM-HBcAb(+) (10/02) HBV-DNA(+)、HBsAg(−)、HBeAg(−)、HBeAb(+) (10/02) HBV-DNA(+) (10/02) HBV-DNA(+) (10/03) HBV-DNA(+)、HBeAg(−)、HBeAb(±) (10/04) HBV-DNA(−)、HBeAg(−)、HBeAb(±) (10/07) HBV-DNA(−)、HBeAg(−)、HBeAb(±) (10/11) HBV-DNA(−)、HBsAb(−)、HBeAg(−)、HBeAb(±) (11/01) HBV-DNA(−)、HBsAg(−)、HBsAb(−)、HBeAg(−)、HBeAb(±) (11/04) HBV-DNA(−)、HBsAg(−)、HBsAb(−) HBeAg(−)、HBeAb(±) (11/06) HBV-DNA(−)、HBsAg(−)、HBsAb(−) HBcAb(+)、HBeAg(−)、HBeAb(±) (11/09) HBsAb(−)、HBeAg(−)、HBeAb(±) (11/10)	-	HBV-DNA(−) HBsAg(−) HBsAb(−) HBcAb(+) (11/09)	陽性(輸血後の10年2～3月時点) 陰性(輸血後の11年9月時点)	HBV-DNA(+)		本症例は2011年3月1日に未完了報告を行った症例(識別番号 1-10000101)と同一献血者によるものである。 【献血者陽転化情報】 (前回) 08年9月26日HBV関連検査陰性 個別HBV-NAT 陰性(遡及対象) (当該) 09年2月3日HBV関連検査 陰性 個別HBV-NAT 陽性(遡及対象) (1回後) 09年10月5日HBV関連検査陰性 個別HBV-NAT 陽性(遡及対象) (2回後) 10年2月3日HBV関連検査陰性 個別HBV-NAT 陰性(識別番号 1-10000101) (3回後) 10年10月12日HBV関連検査陰性 個別HBV-NAT 陽性(遡及対象) (4回後) 11年2月3日スクリーニングHBV NAT 陽性(陽転献血) また当該前後の献血について、3本の原料血漿、1本の新鮮凍結血漿-LR、4本の赤血球濃厚液-LRを製造。原料血漿1本は確保済み、2本は使用済み。新鮮凍結血漿-LR 1本は医療機関へ供給済み。赤血球濃厚液-LR 4本は医療機関へ供給済み。 担当医より「輸血血液と感染症の因果関係はあると考える」とのコメントが得られた。	2単位	備考欄参照	1本の照射赤血球濃厚液-LRを製造。	赤血球製剤は医療機関へ供給済み(輸血による感染は不明であるとの担当医の見解を得ている)。	重篤	軽快		献血者スクリーニングNAT陽転に係る遡及調査により特定された症例。なお、同一献血者の2回後献血(10年2月3日)由来の製剤について医療機関から企業に報告があり、2011年3月1日付けで感染症報告が行われた。 患者検体と献血者検体(HBV陽性)とのHBV塩基配列の相同性の比較は、患者検体がHBV-DNA陰性であったため、実施できなかった。

日赤番号	識別番号	FAX受付日	報告受領日	販売名(一般名)	患者性別	年代	原疾患	感染症名	投与年月	投与前検査(年月)	投与後検査(年月)	日赤投与前検査	日赤投与後検査	受血者個別NAT	献血者個別NAT	併用血液製剤等	備考	使用単位数	供血者再献血※	同一供血者製剤確保※	同一供血者製剤使用※	感染症等転帰	転帰	供血者発遡及の場合の供血者保管検	供血者発遡及の場合の供血者の検査値
3-1100082	A-11000072	2011/11/15	2011/11/28	照射濃厚血小板-LR(人血小板濃厚液(放射線照射)) 赤血球濃厚液-LR(人赤血球濃厚液) 新鮮凍結血漿-LR成分採血(新鮮凍結人血漿)	女	30	生殖器腫瘍		11/07	HBsAg(−) HBsAb(−) HBcAb(−) IgM-HBcAb(−) (11/07)	HBsAg(+) HBsAb(−) HBcAb(−) IgM-HBcAb(−) (11/11) HBV-DNA(+) HBsAg(+) HBsAb(−) HBcAb(−) HBeAg(+) HBeAb(−) (11/11)	HBsAb(-) HBcAb(-) (11/7)	HBV-DNA(+) HBsAg(-) HBsAb(-) HBcAb(+) (11/11)	陽性(輸血後)	保管検体11本HBV-DNA(-)、1本HBV-DNA(+)		HBV-DNA陽性輸血用血液(献血者)情報 ①同一採血番号製剤:1本の赤血球濃厚液-LRを製造、医療機関へ供給済み。 ②供血者再来献血:3回献血に再来(HBV関連検査陰性) 3本の原料血漿、1本の濃厚血小板-LR、1本の赤血球濃厚液-LRを製造。原料血漿は全て確保済み。濃厚血小板-LR、・赤血球濃厚液-LRは医療機関へ供給済み ③当該以前の献血:可能な限り過去に遡り、保管検体の個別NATが陰性と判定されるまですべての輸血用血液・原料血漿を遡及する。 担当医より「輸血血液と感染症の因果関係はあると考える」とのコメントが得られた。	20単位 10単位 10単位	5/12(HBV関連検査陰性)	5本の原料血漿、2本の新鮮凍結血漿-LR、5本の赤血球濃厚液-LRを製造。原料血漿はすべて確保済み。新鮮凍結血漿-LRはすべて確保済み。	赤血球濃厚液-LRはすべて医療機関へ供給済み。	重篤	未回復		献血者検体(当該採血検体PCR増幅できなかったため、2011年5月23日採血検体を用いた。)と患者検体とでPreS/S領域を含むP領域の前半部の1550bpの塩基配列を比較したところ、1箇所の相違が見られ、他はすべて一致した。両者のHBV-DNAはGenotypeBで塩基配列からSubtypeはadwと推定した。
3-1100092	A-11000085	2011/12/13	2011/12/26	照射濃厚血小板-LR(人血小板濃厚液(放射線照射))	男	70	血液疾患		11/09	HBsAg(−) (11/07) HBsAb(−) HBcAb(−) (11/07) HBV-DNA(−) HBsAg(−) HBsAb(−) HBcAb(−) (11/09) (遡及調査情報提供にて実施)	HBV-DNA(+) HBsAg(+) HBsAb(−) HBcAb(−) (11/11)(遡及調査情報提供にて実施) HBV-DNA(+) HBsAg(+) HBsAb(−) HBcAb(−) (11/12)(遡及調査情報提供にて実施)	HBV-DNA(−) HBsAg(−) HBsAb(−) HBcAb(−) (11/09)	HBV-DNA(+) HBsAg(+) HBsAb(−) HBcAb(−) (11/11) HBV-DNA(+) HBsAg(+) HBsAb(−) HBcAb(−) (11/12)	陰性(輸血前) 陽性(輸血後)	保管検体1本についてHBV-DNA(+)		【献血者陽転化情報】 当該献血 2011年9月20日 HBV関連検査 陰性 個別HBV-NAT 陽性(遡及調査) 次回献血 2011年11月11日 HBs抗原検査陽性(陽転献血) 個別HBV-NAT 陽性(遡及調査)	10単位	-	1本の原料血漿を製造、確保済み。	-	重篤	未回復		患者検体と献血者検体とでPreS/S領域を含むP領域の前半部の1550bpの塩基配列を比較したところ、すべて一致した。両者のHBウイルスはGenotypeCで塩基配列からSubtypeはadwと推定した。

日赤番号	識別番号	FAX受付日	報告受領日	販売名(一般名)	患者性別	年代	原疾患	感染症名	投与年月	投与前検査(年月)	投与後検査(年月)	日赤投与前検査	日赤投与後検査	受血者個別NAT	献血者個別NAT	併用血液製剤等	備考	使用単位数	供血者再献血※	同一供血者製剤確保※	同一供血者製剤使用※	感染症等転帰	転帰	供血者発遡及の場合の供血者保管検	供血者発遡及の場合の供血者の検査値
陽転事例																									
3-1100079	A-11000069	2011/11/2	2011/11/15	照射赤血球濃厚液−LR(人赤血球濃厚液(放射線照射))	女	80	糖尿病腎・泌尿器系疾患その他の疾患	B型肝炎	11/05	HBsAg(−) HBsAb(−) HBcAb(−) (11/05)	HBsAg(+) (11/10) HBV-DNA(+) HBcAb(+) IgM-HBcAb(+) HBeAg(+) (11/10)	HBV-DNA(−) HBsAg(−) HBsAb(−) HBcAb(−) (11/05)	HBV-DNA(+) HBsAg(+) HBsAb(−) HBcAb(+) (11/10) HBV-DNA(+) HBsAg(+) HBsAb(−) HBcAb(+) (11/10)	陰性(輸血前) 陽性(輸血後)	保管検体2本(全部)についてHBV-DNA(-)			3単位	0/2	1本の原料血漿、1本の新鮮凍結血漿-LRを製造、どちらも確保済み。	-	非重篤	未回復		
3-1100080	A-11000070	2011/11/2	2011/11/15	赤血球濃厚液-LR(人赤血球濃厚液) 新鮮凍結血漿−LR成分採血(新鮮凍結人血漿)	女	30	生殖器腫瘍	B型肝炎	11/06	HBsAg(−) HBsAb(−) HBcAb(−) (11/06)	HBV-DNA(+) HBsAg(−) (11/10)	HBV-DNA(−) HBsAg(−) HBsAb(−) HBcAb(−) (11/06)	HBV-DNA(−) HBsAg(−) HBsAb(−) HBcAb(−) (11/11)	陰性(輸血前) 陽性(輸血後の11年10月点) 陰性(輸血後の11年11月時点)	保管検体9本(全部)についてHBV-DNA(-)		担当医より「輸血血液と感染症の因果関係は不明である」とのコメントが得られた。	12単位 15単位	2/9(HBV関連検査陰性)	1本の原料血漿、5本の新鮮凍結血漿-LRをい製造。原料血漿は確保済み。新鮮凍結血漿-LRは全て確保済み。	-	非重篤	不明		
3-1100090	A-11000082	2011/12/12	2011/12/22	照射赤血球濃厚液−LR(人赤血球濃厚液(放射線照射))	男	70	血液疾患	B型肝炎	11/07-10	HBsAg(−) (11/04) HBsAb(−) HBcAb(−) (11/07)	HBV-DNA(+) (11/11)	HBV-DNA(−) HBsAg(−) HBsAb(−) HBcAb(−) (11/07)	HBV-DNA(+) HBsAg(+) HBsAb(−) HBcAb(−) (11/12)	陰性(輸血前) 陽性(輸血後)	保管検体3本全部についてHBV-DNA(-)			6単位	0/3	3本の新鮮凍結血漿-LRを製造、すべて確保済み。	-	非重篤	未回復		

日赤番号	識別番号	FAX受付日	報告受領日	販売名(一般名)	患者性別	年代	原疾患	感染症名	投与年月	投与前検査(年月)	投与後検査(年月)	日赤投与前検査	日赤投与後検査	受血者個別NAT	献血者個別NAT	併用血液製剤等	備考	使用単位数	供血者再献血※	同一供血者製剤確保※	同一供血者製剤使用※	感染症等転帰	転帰	供血者発遡及の場合の供血者保管検体(抗	供血者発遡及の場合の供血者の検査値
3-1100093	A-11000086	2011/12/16	2011/12/27	照射濃厚血小板-LR(人血小板濃厚液(放射線照射)) 照射赤血球濃厚液－LR(人赤血球濃厚液(放射線照射))	男	10	先天性疾患	B型肝炎	11/01-06 11/02-04	以前にも頻回に輸血実施。 10/02～血小板製剤2パック/週 輸血継続。 HBsAg(－)(10/12) HBV-DNA(－) HBsAg(－) HBsAb(－) HBcAb(－) (11/01)	HBsAg(－)、HBsAb(－)、HBcAb(－)(11/02) HBsAg(－)、HBsAb(－)、HBcAb(－)(11/03) HBV-DNA(－)、HBsAg(－)、HBsAb(－)、HBcAb(－)(11/04) HBV-DNA(－)、HBsAg(－)、HBsAb(－)、HBcAb(－)(11/05) HBV-DNA(＋)、HBsAg(－)、HBsAb(－)、HBcAb(－)(11/06) HBsAg(－)、HBsAb(－)、HBcAb(－)(11/06) HBV-DNA(＋)、HBsAg(－)、HBsAb(－)、HBcAb(－)(11/07) HBsAg(＋)、HBsAb(－)、HBcAb(－)(11/07) HBsAg(＋)、HBsAb(－)、HBcAb(－)(11/08) HBsAg(＋)、HBsAb(－)、HBcAb(－)(11/09) HBsAg(＋)、HBsAb(－)、HBcAb(－)(11/10) HBsAg(＋)、HBsAb(－)、HBcAb(－)(11/11) HBsAg(＋)、HBsAb(－)、HBcAb(－)(11/12) (この結果を受けて過去に遡って検査実施)	HBV-DNA(－) HBsAg(－) HBsAb(＋) HBcAb(－) (11/01)	HBV-DNA(＋) HBsAg(＋) HBsAb(－) HBcAb(－) (11/08)	陰性(輸血前) 陽性(輸血後)	保管検体57本全部についてHBV-DNA(-)			470単位 20単位	37/56＊(HBV関連検査陰性)＊被疑薬は57本	34本の原料血漿、2本の新鮮凍結血漿-LRを製造。原料血漿は5本確保済み。新鮮凍結血漿-LRは1本確保済み。	原料血漿は29本使用済み。新鮮凍結血漿-LR1本は医療機関へ供給済みで未使用であり回収済み。	非重篤	不明		
				輸血によるHCV感染報告例(疑い例を含む。)																					
供血者陽性事例																									
(該当例なし)																									
陽転事例																									
3-1100081	A-11000071	2011/11/7	2011/11/17	照射赤血球濃厚液－LR(人赤血球濃厚液(放射線照射)) 照射濃厚血小板-LR(人血小板濃厚液(放射線照射))	女	70	循環器疾患	C型肝炎	11/07	HCV-Ab(－)(11/07)	HCV-Ab(＋)(11/10) HCV-RNA(＋)(genotype 1b)(11/11)	HCV-RNA(－) HCV-Ab(－) (11/07)	HCV-RNA(＋) HCV-Ab(＋) (11/10)	陰性(輸血前) 陽性(輸血後)	保管検体4本全部についてHCV-RNA(-)			3単位 10単位	2/4(HCV関連検査陰性)	4本の原料血漿を製造、全て確保済み。	－	重篤	未回復		

日赤番号	識別番号	FAX受付日	報告受領日	販売名(一般名)	患者性別	年代	原疾患	感染症名	投与年月	投与前検査(年月)	投与後検査(年月)	日赤投与前検査	日赤投与後検査	受血者個別NAT	献血者個別NAT	併用血液製剤等	備考	使用単位数	供血者再献血※	同一供血者製剤確保※	同一供血者製剤使用※	感染症等転帰	転帰	供血者発遡及の場合の供血者保管検体(抗	供血者発遡及の場合の供血者の検査値
3-1100083	A-11000073	2011/11/16	2011/11/29	赤血球濃厚液－LR(人赤血球濃厚液)	男	80	消化器腫瘍	C型肝炎	11/06	HCV-Ab(－)(11/05)	HCV-Ab(＋)(11/10) HCV-Ab(＋) HCV-RNA(+)(genotype 1b)(11/11)	－	HCV-RNA(＋) HCV-Ab(＋) (11/11)	陽性(輸血後)	保管検体3本全部についてHCV-RNA(-)			6単位	0/3	2本の原料血漿、1本の新鮮凍結血漿-LRを製造。原料血漿はすべて確保済み。新	－	中等度	未回復		
3-1100084	A-11000074	2011/11/17	2011/11/29	照射赤血球濃厚液－LR(人赤血球濃厚液(放射線照射))	女	90	肝・胆・膵腫瘍	C型肝炎	11/03-09	HCV-Ab(－)(11/02)	HCV-Ab(－)(11/07) HCV-Ab(－)(11/08) HCV-RNA(＋) HCV-Ab(＋) (11/09)	－	HCV-RNA(-) HCV-Ab(-) (11/4) HCV-RNA(-) HCV-Ab(-) (11/6) HCV-RNA(＋) HCV-Ab(-) (11/7) HCV-RNA(＋) HCV-Ab(-) (11/8)	陽性(輸血後)	保管検体16本全部についてHCV-RNA(-)		患者は胆管癌にて死亡、剖検無し。死亡と本剤の関連性無し(担当医の見解)	19単位	0/16	11本の原料血漿、5本の新鮮凍結血漿-LRを製造。原料血漿は9本確保済み。新鮮凍結血漿-LRはすべて確保済み。	原料血漿2本使用済み	非重篤	死亡		
3-1100087	A-11000079	2011/12/6	2011/12/20	照射赤血球濃厚液－LR(人赤血球濃厚液(放射線照射))	女	80	その他の疾患	C型肝炎	11/10	HCV-Ab(－)(11/10)	HCV-Ab(－)(11/11) HCV-Ab(＋)(11/12)	－	HCV-RNA(＋) HCV-Ab(＋) (11/12)	陽性(輸血後)	保管検体1本全部についてHCV-RNA(-)			2単位	0/1	1本の原料血漿を製造、確保済み。	－	重篤	軽快		
3-1100088	A-11000080	2011/12/7	2011/12/20	照射赤血球濃厚液－LR(人赤血球濃厚液(放射線照射))	女	80	消化器疾患	C型肝炎	11/4	HCV-Ab(－)(11/03)	HCV-Ab(＋)(11/11) HCVコア抗原(＋)(11/11)	－	HCV-RNA(＋) HCV-Ab(＋) (11/11) HCV-RNA(＋) HCV-Ab(＋) (11/12)	陽性(輸血後)	保管検体2本全部についてHCV-RNA(-)			2単位	0/2	2本の原料血漿を製造、1本は確保済み。	原料血漿1本は使用済み。	重篤	未回復		
3-1100089	A-11000081	2011/12/8	2011/12/21	照射濃厚血小板-LR(人血小板濃厚液(放射線照射)) 照射赤血球濃厚液－LR(人赤血球濃厚液(放射線照射))	男	60	血液腫瘍その他の疾患	C型肝炎	10/12-11/04	HCV-Ab(－)(10/10)	HCV-RNA(＋) HCV-Ab(＋) (11/11)	HCV-RNA(－) HCV-Ab(－) (10/12)	HCV-RNA(＋) HCV-Ab(－) (11/11)	陰性(輸血前) 陽性(輸血後)	保管検体41本全部についてHCV-RNA(-)			240単位 24単位	25/40(HCV関連検査陰性)	37本の原料血漿、4本の新鮮凍結血漿-LRを製造。原料血漿は15本確保済み。	原料血漿は22本使用済み。新鮮凍結血漿-LRはすべて医療機関へ供給済み。	軽微	不明		

日赤番号	識別番号	FAX受付日	報告受領日	販売名（一般名）	患者性別	年代	原疾患	感染症名	投与年月	投与前検査(年月)	投与後検査(年月)	日赤投与前検査	日赤投与後検査	受血者個別NAT	献血者個別NAT	併用血液製剤等	備考	使用単位数	供血者再献血※	同一供血者製剤確保※	同一供血者製剤使用※	感染症等転帰	転帰	供血者発遡及の場合の供血者保管検体（抗	供血者発遡及の場合の供血者の検査値
3-1100091	A-11000084	2011/12/13	2011/12/26	照射赤血球濃厚液－LR(人赤血球濃厚液(放射線照射))	女	80	その他の疾患	C型肝炎	11/02	HCV-Ab（－） (11/02)	HCV-Ab（＋） (11/12)	調査中	調査中	調査中	保管検体1本全部についてHCV-RNA(-)			2単位	0/1	調査中	調査中	重篤	未回復		
				輸血によるHIV感染報告例（疑い例を含む。）																					
（該当例なし）																									
				輸血によるサイトメガロウイルス感染報告例（疑い例を含む。）																					
陽性等事例																									
3-1100086	A-11000078	2011/12/1	2011/12/14	新鮮凍結血漿-LR(新鮮凍結人血漿) 照射濃厚血小板-LR((人血小板濃厚液(放射線照射)) 赤血球濃厚液－LR(人赤血球濃厚液)	女	～10	先天性疾患	サイトメガロウイルス感染	11/09-10	11/09 双胎間輸血症候群受血児。	サイトメガロウイルスpp65抗原陽性 6/15万 (11/11) 尿中ウイルス分離（蛍光抗体法、無数/75μL）、咽頭ぬぐい液ウイルス分離（－） (11/11)	-	IgM-CMV-Ab(+)	-	保管検体10本（全部）CMV-DNA(-) IgM-CMV-Ab(-) IgG-CMV-Ab(+)			10単位 10単位 6単位	-	4本の原料血漿、1本の新鮮凍結血漿-LR, 5本の赤血球濃厚液-LRを製造。	(CMV感染症例のため製剤の確保・使用状況は記載しない)	重篤	未回復		
				輸血による細菌等感染報告例（疑い例を含む。）																					
陽性等事例																									
3-1100076	A-11000065	2011/10/19	2011/11/1	照射赤血球濃厚液－LR(人赤血球濃厚液(放射線照射))	男	80	血液疾患	細菌感染	11/10	-	輸血前後で著変なし。WBC 2600/μL、CRP 1.32mg/dL 翌日（26時間後） 悪寒あり。BT 40℃、WBC 3300/μL、CRP 1.03mg/dL 患者血培よりAeromonas sobriaを検出。	当該製剤のセグメントチューブ（1本）で細菌培養試験を実施、陰性。	-	-	-		被疑薬：採血14日目の照射赤血球濃厚液-LR(3本)	2単位	-	1本の原料血漿を製造、確保済み。	-	重篤	軽快		

平成24年 3月14日開催
薬事・食品衛生審議会
運営委員会提出資料

別紙

日本赤十字社

試行的HEV20プールNAT実施状況について

（輸血後HEV感染の予防対策）

北海道赤十字血液センター管内
調査期間：平成17年1月1日～平成23年12月31日

	献血者数	HEV-RNA陽性	陽性率
H17. 1～H18. 2[*1]	341, 174	45	1／7, 582
H18. 3～H23. 12[*2]	1, 590, 673	186	1／8, 552
合計	1, 931, 847	231	1／8, 363

*1 北海道センターにてNAT実施（ALT高値、検査不合格検体も含む）
*2 血漿分画センターにてNAT実施（ALT高値、検査不合格検体は除く）

事務連絡
平成２４年1月２４日

日本赤十字社血液事業本部　御中

薬事・食品衛生審議会血液事業部会事務局
厚生労働省医薬食品局血液対策課

血液製剤に関する報告事項について

血液事業の推進に御努力いただき、厚く御礼申し上げます。

さて、標記につきましては、平成２３年１１月１８日付け血安第４２０号にて貴社から報告を頂いたところですが、平成２４年３月１４日（水）に平成２３年度第４回血液事業部会運営委員会が開催されますので、下記の事項について資料を作成いただき、平成２４年２月７日（火）までに当事務局あて御提出いただきますようお願いします。記の３については、平成２３年度第３回血液事業部会運営委員会提出資料を更新のうえ、再度御提出ください。

なお、資料の作成に当たっては、供血者、患者及び医療機関の名称並びにこれらの所在地又はこれらの事項が特定できる情報を記載しないよう、個人情報及び法人情報の保護に特段の御配慮をお願いします。

記

１．平成１９年２月２０日付けで報告された輸血用血液製剤でＨＢＶ（Ｂ型肝炎ウイルス）感染が疑われる事例について、残る１人の供血者のその後の検査結果。来訪がなければ、その旨。

２．平成２１年１１月２０日付けで報告された輸血用血液製剤でＨＢＶ（Ｂ型肝炎ウイルス）感染が疑われる事例について、残る２人の供血者のその後の検査結果。来訪がなければ、その旨。

３．試行的ＨＥＶ２０プールＮＡＴについて、その後の調査実施状況。

血安第４２号
平成２４年２月７日

厚生労働省医薬食品局血液対策課長 様

日本赤十字社
血液事業本部長

血液製剤に関する報告事項について（回答）

平成24年1月24日付事務連絡によりご依頼のありました標記の件について、下記のとおり資料を作成しましたので報告いたします。

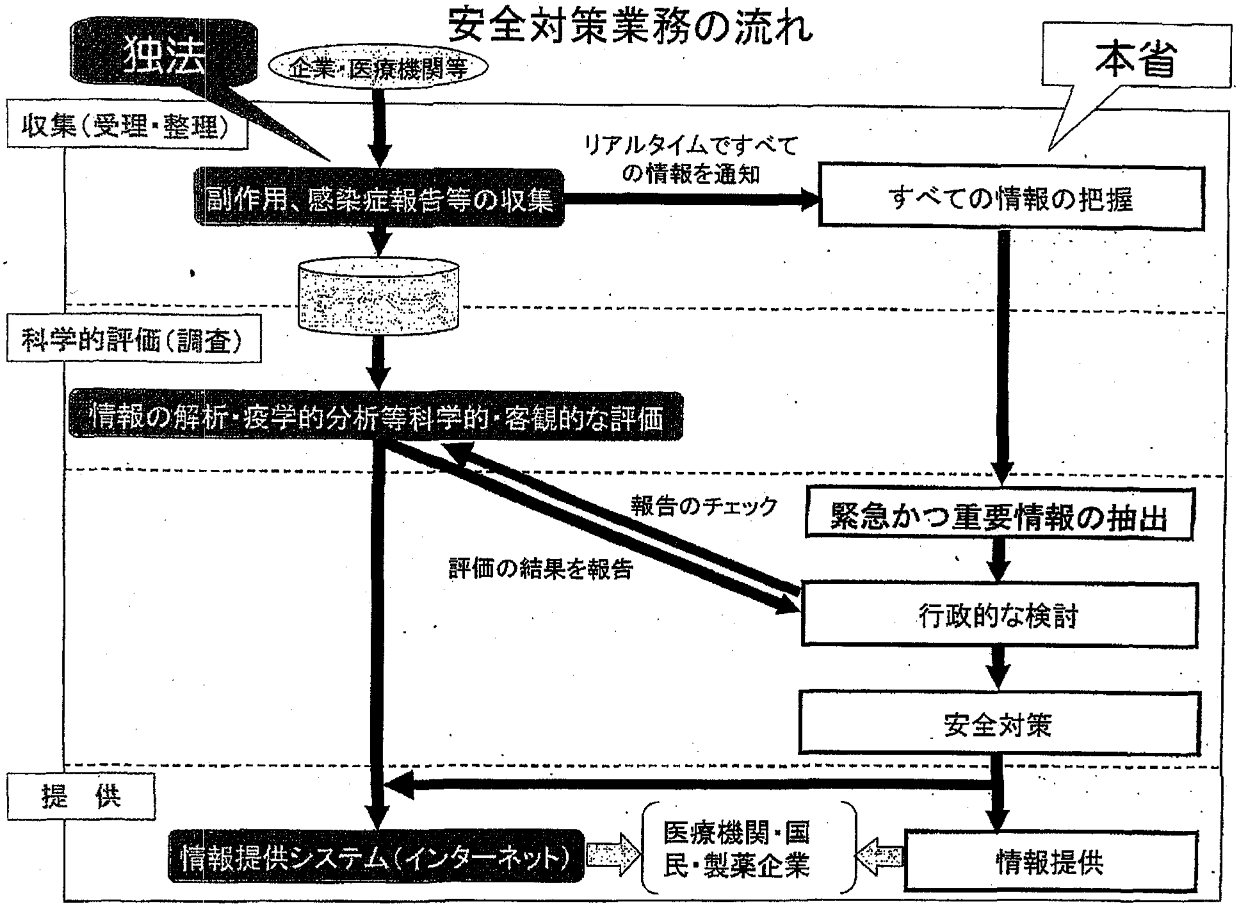
記

１．平成19年2月20日付けで報告された輸血用血液製剤でＨＢＶ（Ｂ型肝炎ウイルス）感染が疑われる事例について、残る1人のその後の来訪なし。(3名中2名が来所、検査は全て陰性)

２．平成21年11月20日付けで報告された輸血用血液製剤でＨＢＶ（Ｂ型肝炎ウイルス）感染が疑われる事例について、残る2人のその後の来訪なし。(23名中21名が来所、検査は全て陰性)

３．試行的ＨＥＶ２０プールＮＡＴについて、その後の調査実施状況については別紙のとおり。

安全対策業務の流れ



独法

企業・医療機関等

本省

収集（受理・整理）

副作用、感染症報告等の収集

リアルタイムですべての情報を通知

すべての情報の把握

科学的評価（調査）

情報の解析・疫学的分析等科学的・客観的な評価

報告のチェック

評価の結果を報告

緊急かつ重要情報の抽出

行政的な検討

安全対策

提　供

情報提供システム（インターネット）

医療機関・国民・製薬企業

情報提供

資料３－３

献血件数及びＨＩＶ抗体・核酸増幅検査陽性件数

年	献血件数 （検査実施数）	陽性件数 （ ）内女性 ［ ］内核酸増幅検査のみ陽性	10万件当たり
	件	件	件
１９８７年　（昭和 62 年）	８,２１７,３４０	１１(1)	0.134
１９８８年　（昭和 63 年）	７,９７４,１４７	９(1)	0.113
１９８９年　（平成 元年）	７,８７６,６８２	１３(1)	0.165
１９９０年　（平成 2 年）	７,７４３,４７５	２６(6)	0.336
１９９１年　（平成 3 年）	８,０７１,９３７	２９(4)	0.359
１９９２年　（平成 4 年）	７,７１０,６９３	３４(7)	0.441
１９９３年　（平成 5 年）	７,２０５,５１４	３５(5)	0.486
１９９４年　（平成 6 年）	６,６１０,４８４	３６(5)	0.545
１９９５年　（平成 7 年）	６,２９８,７０６	４６(9)	0.730
１９９６年　（平成 8 年）	６,０３９,３９４	４６(5)	0.762
１９９７年　（平成 9 年）	５,９９８,７６０	５４(5)	0.900
１９９８年　（平成 10 年）	６,１３７,３７８	５６(4)	0.912
１９９９年　（平成 11 年）	６,１３９,２０５	６４(6)	1.042
２０００年　（平成 12 年）	５,８７７,９７１	６７(4) [3]	1.140
２００１年　（平成 13 年）	５,７７４,２６９	７９(1) [1]	1.368
２００２年　（平成 14 年）	５,７８４,１０１	８２(5) [2]	1.418
２００３年　（平成 15 年）	５,６２１,０９６	８７(8) [2]	1.548
２００４年　（平成 16 年）	５,４７３,１４０	９２(4) [2]	1.681
２００５年　（平成 17 年）	５,３２０,６０２	７８(3) [2]	1.466
２００６年　（平成 18 年）	４,９８７,８５７	８７(5) [1]	1.744
２００７年　（平成 19 年）	４,９３９,５５０	１０２(3) [6]	2.065
２００８年　（平成 20 年）	５,０７７,２３８	１０７(3) [0]	2.107
２００９年　（平成 21 年）	５,２８７,１０１	１０２(6) [2]	1.929
２０１０年　（平成 22 年）	５,３１８,５８６	８６(3) [1]	1.617
２０１１年　（平成 23 年） （１～１２月）	５,２５２,１８２ （速報値）	８９(8) [3]	1.695

（注１）・ 昭和６１年は、年中途から実施したことなどから、3,146,940 件、うち、陽性件数１１件（女性０）となっている。
（注２）・ 抗体検査及び核酸増幅検査陽性の血液は廃棄され、製剤には使用されない。
・ 核酸増幅検査については、平成１１年１０月より全国的に実施している。
（注３）・ 平成２３年は、１月～１２月の速報値で集計している。

ＨＩＶ抗体・核酸増幅検査陽性献血者数内訳

1. 性別･年齢区分･国別

	男性			女性			合計		
	日本人	外国人	計	日本人	外国人	計	日本人	外国人	計
	人	人	人	人	人	人	人	人	人
16～19歳	36	1	37	11	0	11	47	1	48
20～29歳	545	30	575	49	4	53	594	34	628
30～39歳	520	13	533	26	2	28	546	15	561
40～49歳	186	1	187	12	1	13	198	2	200
50～69歳	84	0	84	7	0	7	91	0	91
合計	1371	45	1416	105	7	112	1476	52	1528

※ 昭和61年～平成23年12月（昭和61年については年途中から集計）

2．都道府県別（献血地別）

県別	61年	62年	63年	元年	2年	3年	4年	5年	6年	7年	8年	9年	10年	11年	12年	13年	14年	15年	16年	17年	18年	19年	20年	21年	22年	23年	合計	構成割合	ブロック別 陽性献血件数	構成割合
	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(%)	(件)	(%)
1.北海道			1			1	2	1	1	1		1	1	3	2	2	3	2	2	3	2	3	3	2	2	2	40	2.6		
2.青森			2									1					1	1	2	1	1	1		1	2		13	0.9		
3.岩手										1				1										3		1	6	0.4		
4.宮城							1	1				1	1	1		1	1	1	1	2				2			13	0.9	北海道	
5.秋田																1			1				1		1		4	0.3	・東北	
6.山形													1				1							1	1		4	0.3		
7.福島							1					2		1	1			1					1		1	2	10	0.7	90	5.9
8.茨城					1	1	4	2			1	2		1	2	1	1				1	1	1	5	1	1	26	1.7		
9.栃木					3	1				2	1	1		1		3		1		1	4	2	1	1	1	3	26	1.7		
10.群馬					1	1		1				1		1	3	1		2		3		2	1	3		1	21	1.4		
11.埼玉		1				1	1	2	1	2	3	3	3	3	3	3	3	3	5	2	1	3	2	8	3	1	57	3.7		
12.千葉							1	6	2	2	3	7	2	4	5	4	5	3	3	2	2	6	9	5	6	8	85	5.6	関東	
13.東京	10	6	4	10	10	11	12	11	14	21	18	18	19	27	26	29	23	25	24	22	24	17	21	19	25	18	464	30.4		
14.神奈川		1		1	1	4	1	3	4	2	5	3	4	3	5	3	5	5	8	4	5	5	5	1	2	4	84	5.5	763	49.9
15.新潟				1		1		1						1		2			1		2			2			11	0.7		
16.富山					2						1				1		1				1						6	0.4		
17.石川																2				1		3		1			7	0.5	北陸・	
18.福井			1							2							1	1									5	0.3	甲信越	
19.山梨					1	1					1					1											4	0.3		
20.長野							1	1			2						1				1	1					7	0.5	40	2.6
21.岐阜							1									1			1				1	2	1	2	9	0.6		
22.静岡						1	3		1						1	1		1	1			4		2	1	2	18	1.2	東海	
23.愛知		1			3	2		3	1	1			4	3	2	3	2	2	4	4	5	4	10	4	2	3	63	4.1		
24.三重											1	1	1		1			1		2				1			8	0.5	98	6.4
25.滋賀																1	3						1	2			7	0.5		
26.京都								2		2	1	1				2	5	2		4	5	1			2	5	32	2.1		
27.大阪	1	1	1	1	3		1		4	2	1	8	14	6	8	10	10	15	17	19	17	26	26	13	18	16	238	15.6		
28.兵庫						2			1	2				2	1	1	4	5	3		3	3	4	3	2		36	2.4	近畿	
29.奈良										1	2	3	1		1		1	1	1				1	1			13	0.9		
30.和歌山																			2				1	1		2	6	0.4	332	21.7
31.鳥取									1							1				1			1		1		5	0.3		
32.島根						1															1				3	1	6	0.4		
33.岡山									2									2	1	2	2	3	1			1	14	0.9	中国	
34.広島							2	1	1				1				1	2	6		2	2	1	4		1	24	1.6		
35.山口					1						2												1		1		5	0.3	54	3.5
36.徳島										1				1						1		1	1	1		1	7	0.5		
37.香川							1												1			1	2	2	1		8	0.5	四国	
38.愛媛													1	1	2	3	2	2		1	1	2		1		2	18	1.2		
39.高知																		1	1		1		1	1	1		6	0.4	39	2.6
40.福岡							1		2	2	2	1	1	1	1	2	4	2	2		3	1	3	2	4	4	38	2.5		
41.佐賀																											0	0.0		
42.長崎																	2					1	1	2		2	8	0.5		
43.熊本						1				2	1			1	2		1	2		1	1	2	2	2	1	3	22	1.4		
44.大分									1									2	1								4	0.3	九州	
45.宮崎														2						2		1	1	1		1	8	0.5	・沖縄	
46.鹿児島							1						2			1	1		1		1	1	2		2	1	13	0.9		
47.沖縄		1									1							2	3		1	5	1	3	1	1	19	1.2	112	7.3
合計	11	11	9	13	26	29	34	35	36	46	46	54	56	64	67	79	82	87	92	78	87	102	107	102	86	89	1528	100	1528	100

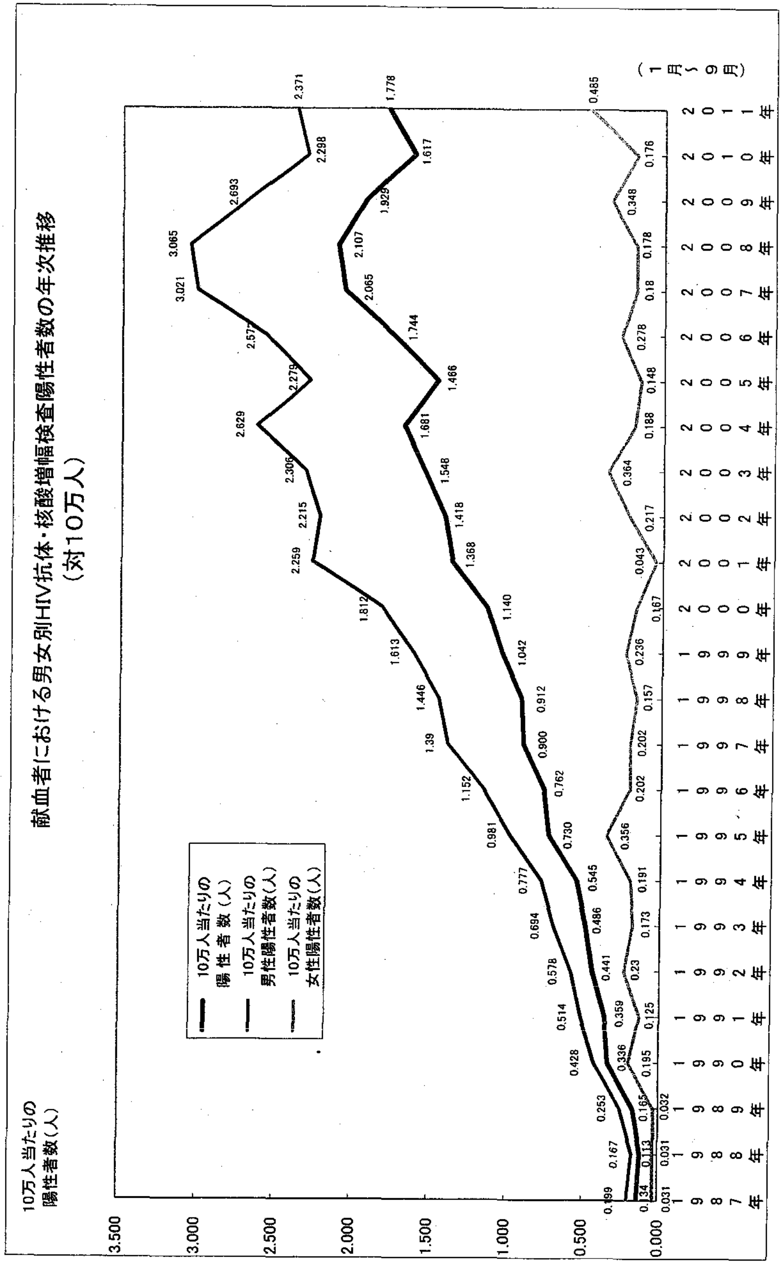
ブロック別HIV抗体・核酸増幅検査陽性献血者

	平成19年			平成20年			平成21年			平成22年			平成23年 (1月～12月)(速報値)		
	献血者	陽性	10万人当たり	献血者	陽性	10万人当たり	献血者	陽性	10万人当たり	献血者	陽性	10万人当たり	献血者	陽性	10万人当たり
北海道 ・東北	人 647,438	件 4	件 0.618	人 651,215	件 5	件 0.768	人 677,073	件 9	件 1.329	人 690,050	件 7	件 1.014	人 645,896	件 5	件 0.774
関東	1,559,391	36	件 2.309	1,621,408	40	件 2.467	1,705,070	42	件 2.463	1,698,561	38	件 2.237	1,649,186	36	件 2.183
北陸・ 甲信越	330,485	4	件 1.210	335,848	0	件 0.000	340,901	3	件 0.880	340,203	0	件 0.000	349,241	0	件 0.000
東海	545,248	8	件 1.467	562,610	11	件 1.955	584,495	9	件 1.540	589,557	4	件 0.678	586,872	7	件 1.193
近畿	807,758	30	件 3.714	833,556	33	件 3.959	863,744	20	件 2.316	876,750	22	件 2.509	873,048	23	件 2.634
中国	316,087	5	件 1.582	316,509	4	件 1.264	329,443	4	件 1.214	330,284	5	件 1.514	324,416	3	件 0.925
四国	161,533	4	件 2.476	166,332	4	件 2.405	173,914	5	件 2.875	176,923	2	件 1.130	176,841	3	件 1.696
九州 ・沖縄	571,610	11	件 1.924	589,760	10	件 1.696	612,461	10	件 1.633	616,258	8	件 1.298	646,682	12	件 1.856
合計	4,939,550	102	件 2.065	5,077,238	107	件 2.107	5,287,101	102	件 1.929	5,318,586	86	件 1.617	5,252,182	89	件 1.695

年齢別HIV抗体・核酸増幅検査陽性献血者

	平成19年			平成20年			平成21年			平成22年			平成23年 (1月～9月)(確定値)		
	献血者	陽性	10万人当たり	献血者	陽性	10万人当たり	献血者	陽性	10万人当たり	献血者	陽性	10万人当たり	献血者	陽性	10万人当たり
16才～19才	人 324,414	件 5	1.541	人 308,019	件 2	0.649	人 295,811	件 3	1.014	人 292,853	件 5	1.707	人 202,048	件 1	0.495
20才～29才	人 1,135,102	38 (2)	3.348	人 1,141,746	41	3.591	人 1,139,991	37 (1)	3.246	人 1,080,385	21 (1)	1.944	人 788,180	30 (3)	3.806
30才～39才	人 1,369,241	35 (1)	2.556	人 1,391,141	50 (1)	3.594	人 1,414,747	42 (3)	2.969	人 1,376,596	43 (1)	3.124	人 1,005,300	28 (1)	2.785
40才～49才	人 1,088,410	17	1.562	人 1,171,449	11 (1)	0.939	人 1,272,397	17 (2)	1.336	人 1,350,490	10	0.740	人 1,029,157	6 (1)	0.583
50才～59才	人 770,663	5	0.649	人 785,280	3 (1)	0.382	人 841,168	3	0.357	人 872,113	6 (1)	0.688	人 652,456	5 (1)	0.766
60才～	人 *251,720*	*2*	*0.795*	人 *279,603*	*0*	*0.000*	人 *322,987*	*0*	*0.000*	人 *346,149*	*1*	*0.289*	人 *259,191*	*0*	*0.000*
合　計	人 4,939,550	102 (3)	2.065	人 5,077,238	107 (3)	2.107	人 5,287,101	102 (6)	1.929	人 5,318,586	86 (3)	1.617	人 3,936,332	70 (6)	1.778

(注)陽性件数の()内女性



献血者における男女別HIV抗体・核酸増幅検査陽性者数の年次推移
(対10万人)

資料4

XMRVに関する文献報告（続報）（平成24年3月14日）

血液事業部会運営委員会委員　岡田　義昭

文献番号	文献名	報告国	要約
1	Oakes B, Qiu X, Levine S, Hackett J Jr, Huber BT. Adv Virol. 2011;2011:854540. Epub 2011 Jul 27. Failure to Detect XMRV-Specific Antibodies in the Plasma of CFS Patients Using Highly Sensitive Chemiluminescence Immunoassays.	米国	平成23年2月18日の運営委員会で紹介した論文の2報目である。1報目の論文では、XMRVの遺伝子が陽性となった検体の全てにマウスのDNAが混入していたことを報告したが、今回は前回用いた検体と同じ血漿（CFS：112、健常人：36）を用いてXMRVのエンベロープ（p15Eとgp70）に対する抗体の有無を高感度化学発光法によって検討した。2検体を除き全て陰性であった。2検体はgp70にのみ弱陽性を示したが、ウエスタンブロット法では陰性であった。この2検体は、同一の供血者（健常人）が日を変えて提供したものであった。前回の報告でマウス DNAのコンタミによってXMRV遺伝子陽性となった検体も抗体は検出されなかった。
2	Robinson MJ, Tuke PW, Erlwein O, et al. Adv Virol. 2011;2011:782353. Epub 2011 Jun 9. No Evidence of XMRV or MuLV Sequences in Prostate Cancer, Diffuse Large B-Cell Lymphoma, or the UK Blood Donor Population.	英国	英国の55例の新鮮な前立腺組織と日本（16例）とインド（20例）からのパラフィン包埋した前立腺切片、10例のパラフィン包埋したBリンパ腫からXMRV、及びマウス白血病ウイルスの遺伝子の検出を行い、インドからの検体からXMRVが2例と MuLVが4例検出されたが、全てマウスの遺伝子が混入していた。また、英国の献血者540名とミニプール400検体（19,200の供血者に相当）からもXMRV、及びマウス白血病ウイルスの遺伝子は検出されなかった。英国の供血者にXMRV、及びマウス白血病ウイルスの感染している証拠は見つけられなかった。
3	Kearney MF, Lee K, Bagni RK, et al. Adv Virol. 2011;2011:272193. Epub 2011 Nov 17. Nucleic Acid, Antibody, and Virus Culture Methods to Detect Xenotropic MLV-Related Virus in Human Blood Samples.	米国	UC-Davisの前立腺癌患者由来の血液108検体からXMRV-RNAとエンベロープとgagに対する抗体の有無、NIHの医療センターの前立腺患者26症例からの血液では XMRVのRNAとDNAの検出、その中の22症例ではエンベロープとgagに対する抗体の有無、さらに12例では培養法によるウイルス検出、また19症例では前立腺組織からのプロウイルDNAの検体を行った。検討した全ての前立腺癌の患者の血液からXMRV感染の証拠は見つけられなかった。
4	Zhou Y, Steffen I, Montalvo L, et al. Transfusion. 2012 Feb;52(2):332-42. Development and application of a high-throughput microneutralization assay: lack of xenotropic murine leukemia virus-related virus and/or murine leukemia virus detection in blood donors.	米国	XMRV由来のエンベロープをもつ pseudotypeウイルスを作製し、これを用いてReno/Tahoe地域の354人の献血者の血漿におけるXMRVとマウス白血病ウイルスに対する中和活性を測定した。6.5%の血漿に中程度の中和活性が認められたが、ウエスタンブロット法では何れの検体からもXMRVに対する抗体は検出できなかった。また、感染性ウイルスの検出や核酸増幅法によってもXMRVやマウス白血病ウイルスは検出できなかった。以上からXMRVのエンベロールを介しての感染を阻害する供血者は存在するが、XMRVやマウス白血病ウイルスに暴露されたことによって生じた抗体ではなく、非特異的な機構による阻害である。
5	Steffen I,Tyrrell D.L,Stein E, et al. PLoS one. 2011 volume 6.issue 11,e27870 No Evidence for XMRV Nucleic Acids, infectious Virus or Anti-XMRV Antibodies in Canadian Patients with chronic Fatigue Syndrome	カナダ	カルガリーとエドモントンに住む58人のCFSと診断された患者と57人の健常者から血液を採取し、PCRによるXMRV及び類似マウス白血病ウイルスの遺伝子の検出、ウイルス培養、XMRVに対する抗体検査を実施したが、全て陰性であった。以上から、CFSはこれらのウイルスと関係ないという結論になった。
6	Karafin MS, Stramer SL. Transfusion. 2012 Feb;52(2):222-5 The scientific method at work: xenotropic murine leukemia virus-related virus is neither a cause of chronic fatigue syndrome nor a threat to the blood supply.	米国	自然現象の理解はデーターに基づいてなされるべきであり、現時点での自然現象の理解は科学的進歩によってより良いものに置き換えられる、というFrancis Baconの哲学を示しながら、XMRVのこれまでも経緯をまとめている。当初、前立腺癌やCFSの原因と関連していると推定され、健常人からもXMRVが検出されたことから血液の供給のリスクになるとされたが、その後の約30の追試では再現性が得られなかった。また、9つの研究施設が参加した同一検体を測定する研究では、これまで陽性例を報告した施設のみ陽性となった。さらに大規模な献血者での調査や保存されていた供血者と受血者の検体を用いた調査などからXMRVは、実験室由来のウイルスのコンタミであるという結果になった。それを受けて ScienceのLombardiらの論文は編集者によって取り下げとなった。さらに PNASに掲載されたLoらの論文は著者らによって取り下げられた。なお、Ian LipkinらのCFS患者からのXMRV検出結果の結論は出されていない。

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文献1

Research Article

Failure to Detect XMRV-Specific Antibodies in the Plasma of CFS Patients Using Highly Sensitive Chemiluminescence Immunoassays

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In 2009, Lombardi et al. reported their startling finding that the gammaretrovirus xenotropic murine leukemia virus-related retrovirus (XMRV) is present in 67% of blood samples of patients suffering from chronic fatigue syndrome (CFS), as opposed to only 3.7% of samples from healthy individuals. However, we and others could not confirm these results, using a nested PCR assay. An alternative to this highly sensitive, but contamination-prone, technique is to measure the serological response to XMRV. Thus, we tested the plasma samples from our cohorts of CFS patients and healthy controls for the presence of XMRV-specific antibodies. Using two novel chemiluminescence immunoassays (CMIAs), we show that none of our samples have any XMRV-reactive antibodies. Taken together with our previous findings, we conclude that XMRV is not present in any human individual tested by us, regardless of CFS or healthy control.

1. Introduction

In 2006, Urisman et al. identified a new gammaretrovirus in prostate cancer samples harboring a mutation in a viral defense gene known as *RNASEL* [1]. This new virus, xenotropic murine leukemia virus-related retrovirus (XMRV), was found to be a close relative to known murine leukemia viruses (MLVs) and was the first documented case of human infection with a xenotropic retrovirus. Although XMRV was originally associated with the mutant variant of the *RNASEL* gene, further research could not confirm this association but did find it in about 10% of prostate cancers [2].

The discovery of a new virus that could infect humans lead Lombardi et al. [3] to test for the virus in patients suffering from chronic fatigue syndrome (CFS). CFS is a disease of unknown etiology that manifests as neurological, immunological, and endocrinological dysfunctions. A wide range of viruses have been investigated in the past as causative agents of CFS; however, findings were mixed, and no conclusive evidence of one virus causing CFS has been implicated [4]. Using a nested polymerase chain reaction (PCR), Lombardi et al. found that blood samples of 68 out of 101 (67%) CFS patients contained the XMRV *gag* sequence, as opposed to only 8 out of 212 (3.7%) samples from healthy individuals [3]. The finding of a virus linked to CFS reignited excitement in the field, leading many laboratories around the world to test for this new virus, but the excitement has been short lived. Although some support linking XMRV or MLVs and CFS has been published [3, 5, 6], it has been overshadowed by reports failing to detect the virus in CFS patients [7–20], including a study done by us.

In our original paper [17], we failed to find an association between CFS patients and XMRV, using PCR technology. However, we did detect some XMRV sequences as well as other MLV sequences in some of our samples. Due to the close relationship between XMRV and MLVs, which are present throughout the mouse genome, we tested all of our samples for mouse DNA using a TaqMan qPCR assay for murine mitochondrial cytochrome oxidase, *cox2* [14], as well as a single PCR assay for the highly abundant intracisternal A-type particle (IAP) long terminal repeat sequence, developed by our group [17]. We found that every sample that contained an XMRV or MLV sequence was also positive for mouse DNA contamination. Although we did not claim that our findings provided a full explanation of the origin of XMRV, we put forward a cautionary tale about the risks of mouse DNA contamination in various common laboratory reagents.

One of the criticisms of our study [17] was that we only used PCR technology to test for the presence of XMRV, while the original paper also included serological analyses [3]. Specifically, some groups have developed novel serological tests utilizing western blots and ELISAs in the search for anti-XMRV antibodies, because the presence of antibodies could not be due to mouse DNA contamination [3, 8, 13, 14, 20, 21]. Recently, two prototype direct format chemiluminescent immunoassays (CMIAs) were developed to detect XMRV-specific antibodies [22]. Both CMIAs utilize a direct assay format in which recombinant p15E or gp70 protein serves as both capture and detection antigens. The assays demonstrated excellent sensitivity, detecting early seroconversion bleeds in XMRV-infected rhesus macaques [22]. Moreover, these assays were also shown to detect specific antibodies to MLVs [22]. In this study, we use these two sensitive CMIAs to screen plasma samples from our blinded cohorts for the presence of XMRV-specific antibodies. No samples from our cohort of over 100 CFS patients were positive in either of these assays, while two samples from the healthy control cohort tested positive in one of the CMIA assays; however, reactivity of these same samples was not confirmed by western blot. Thus, these highly sensitive serological studies have confirmed our prior conclusion that the positive XMRV PCR results were a result of mouse DNA contamination, since no antibodies against XMRV were present.

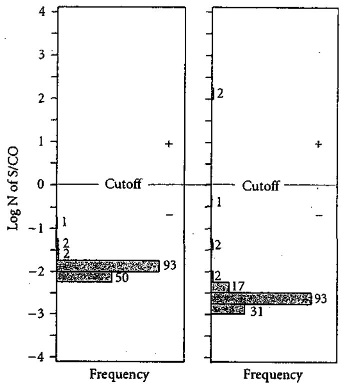
2. Materials and Methods

2.1. Sample Collection. All samples were collected according to the institutional guidelines of Tufts University, after receiving informed consent. The 36 healthy individuals (15 females and 21 males) were recruited on a voluntary basis by the Huber laboratory and were between 18 and 65 years of age. The 112 CFS patients (90 females, 20 males, and 3 unknown), recruited by Dr. Susan Levine, were between 18 and 65 years of age and resided in the Northeastern United States. All patients were diagnosed for CFS according to the CDC criteria, and the majority was completely disabled. The cohort comprised a combination of those with an abrupt and others with a gradual onset of symptoms.

2.2. Preparation of Human Blood and Plasma Samples. Approximately 30 mL of blood were drawn into three heparinized tubes (Becton Dickinson) and shipped overnight (CFS patients) or processed immediately (healthy controls). The blood collection tubes from each individual were consolidated into one 50 mL tube and diluted with PBS, containing CaCl2 and MgCl2 (sigma) at a 1 : 1 ratio. 15 mL of Ficoll (GE Healthcare) was added to two new 50 mL tubes, and 25 mL of the diluted blood was gently layered on top of the Ficoll, followed by a 30 min centrifugation in a Sorvall RT7 plus rotor at 2000 rpm at room temperature. The PBMCs were collected from the interface following the spin and were used for DNA isolation. Ten mL of plasma were also collected from each sample and stored at −80°C. One ml of plasma was sent to Abbott Labs on dry ice overnight for further testing.

2.3. XMRV Chemiluminescent Immunoassays (CMIAs). A detailed procedure can be seen here [22]. Briefly, 100 μL of neat plasma were screened for antibodies to XMRV gp70 and p15E proteins using two prototype ARCHITECT chemiluminescent immunoassays (CMIAs; Abbott Diagnostics, Abbott Park, Ill). The CMIAs utilize a direct assay format in which *E. coli*-expressed XMRV p15E or mammalian-expressed XMRV gp70 were used as both capture and detection antigens. Assay positive controls were derived from XMRV-infected macaque plasmas at 1 : 1000 (PC1) or 1 : 4000 (PC2). A pool of normal human plasma was used as negative control (NC) and as sample diluents. Cutoff (CO) values of the ARCHITECT CMIAs were calculated based on the following formulas: CO = 0.45 × (Calibrator 1 Mean Relative Light Units (RLU)) for p15E CMIA and CO = 0.078 × (Calibrator 2 Mean RLU) for gp70 CMIA. Assay results were reported as the ratio of the sample RLU to the cutoff RLU (S/CO) for each specimen. Specimens with S/CO values <1.00 were considered nonreactive; specimens with S/CO values ≥1.00 were considered initially reactive. The S/CO values of the NC, PC1, and PC2 were 0.16, 12.8, and 3.5 for the gp70 CMIA and 0.13, 7.4, and 2.2 for the p15E CMIA. Initially reactive specimens were retested in duplicate by either ARCHITECT p15E or gp70 CMIAs. Repeatedly reactive specimens were analyzed at 1 : 100 dilution by investigational western blot assays using purified XMRV viral lysate as well as recombinant gp70 protein.

2.4. Western Blot Analysis. Western blot (WB) analysis using purified XMRV viral lysate as well as recombinant gp70 protein was performed as described [22]. Briefly, viral lysate (80 μg/gel) or recombinant gp70 protein (20 μg/gel) were separated by electrophoresis on a 4–12% NuPAGE Bis-Tris 2-dimension gel (Invitrogen, Carlsbad, Calif) in the presence of sodium dodecyl sulfate (SDS). The protein bands on the gel were electrophoretically transferred to a polyvinylidene difluoride (PVDF) membrane (invitrogen). After blocking, the PVDF membrane was cut into 2 mm strips. Strips were incubated with human samples diluted 1 : 100 or XMRV-infected macaque plasma diluted 1 : 200 overnight at 2–8°C. After removal of unbound antibodies, strips were incubated with alkaline phosphatase conjugated goat antihuman IgG (Southern Biotech, Birmingham, Ala) for 30 minutes at room temperature. The strips were washed, and chromogenic substrate solution was added.



(a) p15E CMIA (b) gp70 CMIA

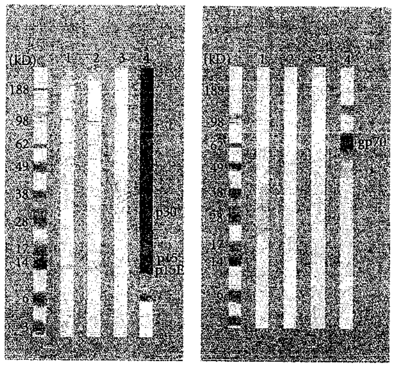
Figure 1: Distribution of p15E CMIA (a) and gp70 CMIA (b) log N of S/CO on 148 samples collected from 112 CFS patients and 36 healthy controls. Numbers of specimens within each log N of S/CO value are shown above the solid bars. Assay cutoffs were equivalent to mean 16 SD and 12 SD for p15E and gp70 CMIAs, respectively, based on blood donor populations [22]. Log N of S/CO, natural log transformation of S/CO.

3. Results

148 blinded plasma samples from our original CFS and healthy control cohorts were analyzed for the presence of XMRV-specific antibodies, using the direct format ARCHITECT p15E and gp70 CMIAs. None of the 148 plasma samples were reactive in the p15E CMIA (Figure 1(a)). Two of the 148 samples (ID = 137, 138) were positive in the gp70 CMIA (Figure 1(b)). Both specimens were weakly reactive in the gp70 CMIA with sample/cut-off (S/CO) values of 7.77 (log N of S/CO = 2.05) and 9.02 (log N of S/CO = 2.20), respectively. Although the samples were repeat reactive in the gp70 CMIA, they were not reactive by WB. As shown in Figure 2, both samples showed no visible WB bands using either XMRV viral lysate proteins (Figure 2(a)) or recombinant gp70 protein (Figure 2(b)). Unblinding of the samples revealed that the two gp70 reactive samples stemmed from two sequential blood collections of a single healthy control (Table 1).

4. Discussion

In our original study, we found no specific relationship between the presence of XMRV and CFS [17]. However, screening the genomic DNA from peripheral blood lymphocytes of both healthy control and CFS cohorts, we did detect PCR products that were identical to XMRV *gag* sequences, as well as other MLV *gag* sequences. Due to the high number of MLV sequences in the mouse genomic DNA, we found it prudent to test for mouse DNA contamination in our samples. Using both a test developed by the Switzer lab at CDC for mouse mitochondrial DNA [14], as well as a test developed by the Coffin lab for the IAP [17], we found that every sample that was positive for XMRV or other MLVs PCR products was also positive for mouse DNA. Although these data provide an explanation for the detection of MLV sequences in our samples, they do not rule out the possibility that XMRV and mouse DNA contamination could be present in the same sample. To clarify this issue, we tested our plasma samples for the presence of XMRV-specific antibodies.



(a) XMRV Viral Lysate (b) Recombinant gp70

Figure 2: WB analysis of gp70 CMIA reactive samples with (a) native XMRV viral proteins and (b) recombinant gp70. WB strip key: 1 & 2: gp70 reactive samples 137 and 138; 3: normal blood donor plasma as negative control; 4: XMRV-infected macaque plasma as positive control. The faint white band in the 65–70 kd region in (B, strips no. 1–3) indicates a lack of specific anti-gp70 antibody.

Recent animal studies showed that XMRV infection elicited a potent humoral immune response in rhesus macaques [22]. The infected macaques developed XMRV-specific antibodies within two weeks of infection and persisted more than 158 days. The predominant responses were to all three structural proteins of XMRV: the envelope protein gp70, the transmembrane protein p15E, and the capsid protein p30 [22]. Sensitivity of both p15E and gp70 CMIAs was validated by the animal model; both CMIAs were able to detect p15E or gp70 specific antibodies as early as day 9 after infection [22]. In contrast, we were unable to detect XMRV p15E or gp70 specific antibodies in the 112 CFS patients and the 36 healthy controls. Although 2 samples from the same healthy control had weak reactivity in gp70 CMIA, the reactivity was not confirmed by recombinant gp70 WB. Furthermore, both samples were nonreactive in p15E CMIA and had no detectable p15E and p30 antibodies by viral lysate WB. Considered in combination with the negative PCR data, the observed isolated and weak gp70 reactivity

Table 1: Results summary for XMRV positive PCR samples. All samples that tested positive for XMRV *gag* sequence in original study [17], as well as the two samples that reacted with the gp70 CMIA, are displayed. Bolded samples showed the VP42 *gag* sequence but did not react with the CMIAs. *The italic data shows the two samples that were reactive in the gp70 CMIA.* CMIA values less than one are considered nonreactive. XMRV GAG: Nested gag PCR. Mcox: murine mitochondrial cytochrome oxidase qPCR. IAP: Intracisternal A-type particle PCR.

ID	Unblinded ID	PCR results XMRV GAG	PCR results Mcox	PCR results IAP	Initial test p15E S/CO	Initial test gp70 S/CO	Repeat test gp70 S/CO
72	TH72.1	+	+	+	0.38	0.06	
128	TH04.1	+	+	+	0.16	0.07	
129	TH01.7	+	+	+	0.15	0.06	
131	TH01.8	+	−	+	0.12	0.06	
132	TH01.3	+	+	+	0.15	0.06	
134	**TH06.1**	+	+	+	**0.15**	**0.07**	
135	*TH01.1*	+	+	+	0.14	*0.09*	
136	TH05.1	+	+	+	0.16	0.06	
137	***TH07.1***	+	+	+	***0.16***	*7.77*	*7.17, 7.21*
138	***TH07.2***	−	−	−	***0.14***	*9.02*	*8.65, 8.77*
143	TH10.1	+	+	+	0.14	0.07	
144	TH11.1	+	−	+	0.14	0.06	
147	TH02.1	+	+	+	0.14	0.07	
152	TH01.5	+	+	+	0.13	0.07	
153	TH21.1	+	+	+	0.15	0.07	
155	TH20.1	+	+	+	0.16	0.06	
156	TH02.2	+	+	+	0.17	0.07	
158	**TH08.1**	+	+	+	**0.13**	**0.07**	
160	**TH03.1**	+	+	+	**0.13**	**0.07**	
161	TH12.1	+	+	+	0.11	0.06	
163	TH19.1	+	+	+	0.16	0.72	0.75, 0.72
164	TH16.1	+	+	+	0.15	0.07	

most likely represents nonspecific reactivity since specificity of the gp70 CMIA was reported as 99.5% [22]. In summary, the serologic data obtained in this study suggests a lack of XMRV infection in our CFS patients and healthy controls. It is theoretically possible that XMRV replicates at very low levels in humans and fails to induce a humoral immune response, or, alternatively, that it is sequestered or latent and specific antibody titers have declined to undetectable levels over time. Although these possibilities cannot be formally excluded, they seem unlikely given responses observed to other human retroviruses. The combination of negative molecular and serologic data do not support an association between CFS and XMRV or other MLVs. Furthermore, the recent demonstration that XMRV is a recombinant of two murine MLVs (23) raises doubts about the validity (24) of the original XMRV claims in CFS (3).

5. Conclusion

With the serological data added to our original finding, we can unequivocally conclude that XMRV is not present in our CFS patient or healthy control cohort samples. Although we have detected XMRV *gag* sequences in three of our samples, they all tested positive for mouse DNA and tested negative for XMRV-specific antibodies. Laboratory mouse strains, as well as wild mice, all carry numerous endogenous MLVs, and extreme caution must be taken when testing for murine-related viruses.

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文献2

Research Article

No Evidence of XMRV or MuLV Sequences in Prostate Cancer, Diffuse Large B-Cell Lymphoma, or the UK Blood Donor Population

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Xenotropic murine leukaemia virus-related virus (XMRV) is a recently described retrovirus which has been claimed to infect humans and cause associated pathology. Initially identified in the US in patients with prostate cancer and subsequently in patients with chronic fatigue syndrome, doubt now exists that XMRV is a human pathogen. We studied the prevalence of genetic sequences of XMRV and related MuLV sequences in human prostate cancer, from B cell lymphoma patients and from UK blood donors. Nucleic acid was extracted from fresh prostate tissue biopsies, formalin-fixed paraffin-embedded (FFPE) prostate tissue and FFPE B-cell lymphoma. The presence of XMRV-specific LTR or MuLV generic *gag*-like sequences was investigated by nested PCR. To control for mouse DNA contamination, a PCR that detected intracisternal A-type particle (IAP) sequences was included. In addition, DNA and RNA were extracted from whole blood taken from UK blood donors and screened for XMRV sequences by real-time PCR. XMRV or MuLV-like sequences were not amplified from tissue samples. Occasionally MuLV *gag* and XMRV-LTR sequences were amplified from Indian prostate cancer samples, but were always detected in conjunction with contaminating murine genomic DNA. We found no evidence of XMRV or MuLV infection in the UK blood donors.

1. Introduction

In 2006, a new gammaretrovirus, xenotropic murine leukaemia virus-related virus (XMRV), was discovered by the Virochip analysis in prostate cancer tissue from patients homozygous for an RNase L mutation [1]. In these patients, the innate antiviral defence RNase L pathway is defective; hence, these patients are likely to be susceptible to viral infection and a population more likely to find a novel virus with disease association in. When a second US study found that 6% of all prostate cancer patients, independent of RNase L mutations, were infected with the virus, thus broadening the population at risk [2], interest in XMRV intensified. However, subsequent studies from the USA [3, 4] and all

European studies [5–7] failed to confirm the presence of XMRV in prostate tissue. More recently it has been suggested that XMRV detection in prostate tissue in the US could be related to the specificity and conditions of the PCR used [8].

In 2009, Lombardi and colleagues reported the presence of XMRV proviral DNA in peripheral blood leucocytes from 3.7% of healthy controls and 67% of patients with chronic fatigue syndrome (CFS) [9]. The detection rate by PCR amplification of XMRV proviral DNA subsequently reduced the estimated CFS prevalence to 7%, with the explanation that RNA extraction and cDNA synthesis had been required to achieve the 67% prevalence originally reported [10]. Lo and colleagues (2010) using predominantly archival material from patients with CFS detected a high prevalence (86.5%) of pMuLVs. These are similar to, but constitute a different group from, the xenotropic endogenous MuLVs to which XMRV belongs [11]. However, questions were raised about how these data were generated [12], and a number of other studies have failed to demonstrate a link between XMRV or pMuLV infection and CFS [13–19].

The causes of B-cell lymphoma are not fully understood [20], but the clinical and epidemiological characteristics are suggestive of the involvement of an infectious agent [21]. Several viruses [22, 23] have been linked to the risk of B-cell lymphoma, most notably EBV [24–26], and retroviruses are implicated in animal leukaemias. Retroviral integration could cause somatic DNA changes leading to clonal expansion of B cells resulting in leukaemia as has been previously described for adult T-cell leukaemia (ATL) and HTLV-1 [27].

The geographical discrepancy of XMRV and pMuLV prevalence remains unexplained. To explore this further, we have tested a variety of tissues from diverse populations; prostate cancer (PC) formalin-fixed paraffin-embedded (FFPE) tissue from Japan and India, fresh prostate tissue samples received from the Urology Clinic at St Mary's Hospital, London, and peripheral blood from English blood donors.

A series of recent papers [28–31] have demonstrated the ease with which specimens can be contaminated with murine DNA sequences. To control for this, all tissue specimens were tested by PCR specific for intracisternal A particle (IAP), a retrotransposon present in multiple copies (∼1000) within the mouse genome [32].

2. Methods and Materials

2.1. Samples and Nucleic Acid Isolation. Prostate biopsies were collected from 55 patients admitted to the Urology Department, St. Mary's Hospital, London, UK to undergo routine biopsy for prostate cancer screening. All patients gave written informed consent for their tissue to be banked for the purposes of research (ethics number 99/CCC/166, August 1999). The DNA was extracted using the QIAamp DNA mini kit (Qiagen, Crawley, UK) following the manufacturer's instructions.

B-cell lymphoma samples were provided by Professor Kikkiri Naresh, Centre for Pathology, Hammersmith Hospital, London, UK. The DNA from 10 Diffuse Large B-cell Lymphoma (DLBLC) patients was extracted from FFPE tissues of lymph nodal or extranodal diffuse large B-cell lymphoma using the DNeasy Blood & Tissue Kit (Qiagen). Briefly, two 15 μm sections were cut and transferred to 1.5 mL Eppendorf tubes. Blades were changed between samples to avoid cross-contamination. Sections were deparaffinised with xylene and ethanol, rehydrated, and incubated with proteinase K and lysis buffer in a shaking water bath at 55°C overnight and the extraction was completed according to the manufacturer's instructions.

Twenty FFPE prostate specimens including 10 prostate cancer (PC) and 10 benign prostatic hyperplasia (BPH) samples were supplied by Professor Ganesh Golpalakrishnan of Vedanayagam Hospital, RS Puram, Coimbatore, India and sixteen specimens from Dr. Takahiro Kimura of the Department of Urology, The Jikei University School of Medicine, Japan. From the Indian blocks, two 10 μM sections were extracted with the QIAamp DNA FFPE tissue kit (Qiagen), according to the manufacturer's instructions. The Japanese samples were provided presliced on glass slides.

Random anonymous whole-blood samples were obtained from the Donation Testing Department at the National Health Service Blood and Transplant (NHSBT) Centre at Colindale, London, UK. Plasma minipools were similarly obtained from NHSBT. All blood and plasma samples were extracted on a Qiagen MDx Biorobot and eluted with 80 μL of Qiagen buffer AVE.

2.2. XMRV, MuLV, and Control Nested PCR. Samples were tested for the presence of XMRV and MuLV proviral DNA using nested PCR, as described previously [14]. Briefly, we used a set of primers that encompasses the 24 bp deletion in the XMRV *gag* leader region, originally described to distinguish XMRV as a new human virus, along with a second set of primers reflecting a sequence conserved amongst most MuLVs. The positive control for the XMRV and MuLV PCRs was plasmid VP62 [1]. The PCR method has been shown to be sensitive enough to pick up one copy of XMRV VP62 plasmid in a background of 500 ng DNA [28]. As a control for sample addition and PCR inhibition, primers to the human beta-globin (hBG) gene were used. DNA extracted from LNCaP (human prostate cancer cells) was used as a positive control for human beta globin. To control for contamination of samples with murine DNA, primers specific to mouse IAP were used as described previously [28]. The positive control for IAP was DNA from the McCoy cell (murine fibroblast cells, ECAAC 90010305). In all PCRs, at least 6 "no template" controls were set up. All PCR products were visualised on Ethidium Bromide-stained 2% agarose gels.

2.3. XMRV, MuLV, and Control Real-Time PCRs for Blood Donor Studies. Real-time PCR was performed as detailed in Table 1. For the proviral DNA analysis, 10 μL of the nucleic acid extract were analysed separately in three individual quantitative PCRs (Q-PCRs).

2.3.1. XMRV Q-PCR and Internal Control. Samples were tested by Q-PCR for XMRV, as described by McCormick et al. [33] and modified as detailed in Table 1. In a Q-PCR to

Table 1: Details of PCRs used to test blood sample.

Sample tested	PCR	Target	Primers and probes	Cycles (N)	Reagents
540 DNAs from whole blood	XMRV Taq Man	gag	XMRV Probe, F, R	60	Qiagen QuantiTect Probe kit
540 DNAs from whole blood	SBCMV Taq Man	SBCMV plasmid	SBCWMVCPF, SBCWMVCPR, SBWMV237F	45	ABgene ABsolute QPCR ROX mastermix
540 DNAs from whole blood	PDH Taq Man	PDH human gene	PDH Probe, F, R	45	ABgene ABsolute QPCR ROX mastermix
600 NAs from whole blood 400 NAs from plasma minipools	XMRV/pMuLV RT Taq Man with BMV RT Taq Man	gag BMV	P2, F3, R4 BMV Probe, F, R	45	Qiagen QuantiTect Probe RT-PCR kit

The TaqMan assay conditions were 15 min at 95°C (15 secs 95°C, 1 min 60°C) $\times N$ cycles. 400 nM concentrations of primers, and 200 nM probes were used in all the TaqMan assays with the exception of the CDC MuLV Taq Man where the concentration of each probe was 100 nM and the PDH TaqMan where the primer concentrations were 50 nM.

control for the extraction efficiency and amplification inhibition coextracted soil-borne cereal mosaic virus (SBCMV) plasmid DNA was used, (5.4×10^6 copies were added to the 33 mL of Qiagen lysis buffer AL used for extracting 96 samples on the MDx Biorobot). This reaction was as described by Ratti et al. [34]. The primer sequences for this reaction were *SBCWMVCPF* (5′-CAC TCA GGA CGG TGA CGA GAT-3′), SBCWMVCPR (5′-GTG ATA CTG TGA GTC TGG TGA TGA TTT-3′) and probe *SBWMV237Fa* (5′ JOE-TTT TGT GAC CTT GGA GGT GAG GCA GTT ATG-BHQ1-3′).

2.3.2. Q-PCR for Quantification of Human DNA. The input of human DNA in each extract was measured by a Q-PCR for the Pyruvate dehydrogenase (PDH) gene. Primers used *PDH Taq 1* (5′-TGA AAG TTA TAC AAA ATT GAG GTC ACT GTT-3′), *PDH Taq 2* (5′- TCC ACA GCC CTC GAC TAA CC -3′) with probe *PDHP* (5′-VIC-CCC CCA GAT ACA CTT AAG GGA TCA ACT CTT AAT TGT-Tamra-3′). Positive control for this reaction was a dilution series of human male DNA (Applied Biosystems, Warrington, UK, Catalogue no. 4312660). The XMRV Q-PCR results were validated when the PDH threshold cycle (Ct) value was greater than the mean Ct minus 3 SD, and the SBCMV control was greater than the mean Ct minus 2 SD. Samples invalid on either control were excluded from the analysis.

2.4. Detection of Gag Sequences by Nested PCR in Blood Donors. Nuclease-free water (Severn Biotech, Kidderminster, UK) was used throughout for the cDNA and PCR mix preparations and as no-template controls. Nucleic acid extracts were tested by nested PCR using the *gag* primers as described by Lombardi et al. [9] and Lo et al. [11], but using Applied Biosystems Taq Gold LD PCR enzyme (Table 1) to overcome the problem of false positives that have arisen from the use of Invitrogen Taq Polymerase [30].

2.5. QRT-PCR Amplification of XMRV/pMuLV in Blood Donors. An XMRV/pMuLV *gag* QRT-PCR assay described by Lo and colleagues [11] but modified to detect the pMuLVs was used to test nucleic acid from whole blood, plasma, and from plasma minipools. Further details of all QPCR and QRT-PCR reactions are listed in Table 1. The primers for this reaction were *F3* (5′-ACC GTT TGT CTC TCC TAA AC-3′) and *R4* (5′-AGG GTA AAG GGC AGA TCG-3′), with probe *P2* (5′-Fam-CCG ACA GCT CCC GTC CTC CCG-Tamra-3′). Nuclease-free water (Severn Biotech) was used throughout for the RT-PCR mix preparations and as no template controls. RT-PCR was performed in a total volume of 50 μL, containing 1x Qiagen QuantiTect RT-PCR buffer and primers, and probes as detailed in Table 1. Synthesis conditions were 50°C for 30 mins, followed by 95°C for 15 mins and 45 cycles of 95°C for 15 secs 60°C for 1 min. Twenty μl of nucleic acid was analysed in a QRT-PCR which multiplexed the XMRV/pMuLV TaqMan with the internal control TaqMan reaction (Brome mosaic virus (BMV)) [35]. The BMV RNA was added to the Qiagen AL lysis buffer and co-extracted with the sample. A sample was valid if the BMV Ct value was greater than the mean Ct minus 2 SD. Samples invalid on the BMV control were excluded from the analysis. The sensitivity of this QRT-PCR was determined as 150 RNA copies/mL (75 viral particles/mL) by calculation from the observed frequency of negatives using the Poisson distribution.

3. Results

3.1. XMRV Detection in Tissue Samples by Nested PCR. A representative stained gel following nested PCR is shown in Figure 1. For routine analysis, 0.11 pg of plasmid DNA (representing approximately 7000 copies/PCR) was used as positive control for XMRV and MuLV. All samples were positive for hBG sequences by PCR. The sensitivity of the IAP PCR has been shown previously to detect as little as 0.0011 pg DNA in a background of 500 ng DNA [28]. The results are summarised in Table 2(a). No evidence of XMRV or MuLV was found in any of the FFPE prostate tissue samples from Japan or the fresh prostate tissues from the UK. Of the 20 Indian samples, four (20%) produced a PCR signal with the MuLV *gag* primers (three prostate cancer, one benign prostatic hyperplasmia) and of these, 2/4 were positive with XMRV LTR primers (both prostate cancer). The IAP PCR was applied to the same samples to see if the positive signal was due to mouse DNA contamination. All MuLV/XMRV amplification was concordant with IAP amplification, except for one prostate cancer sample which was positive for IAP without MuLV/XMRV amplification. Confirmation of murine DNA contamination was achieved using PCR primers specific to mouse mitochondrial DNA (mtDNA). Although this PCR has been shown to be less sensitive than IAP PCR [28], 2/20 of the Indian samples (one prostate cancer, one benign prostatic hyperplasia) were positive for mtDNA. In both of these samples, IAP and MuLV *gag* sequences were amplified. Additionally, one was positive for XMRV (detailed in Table 2(b)).

No evidence of MuLV or XMRV sequences was discovered in the DLBCL samples and none of the DCBCL samples gave an IAP specific product.

Table 2

(a) Amplification from fresh and FFPE tissues by nested PCR

	Fresh prostate tissue	Japan samples	Indian samples	LCBCL samples
Number of cancerous samples	16/55	16	10/20	10
Number of noncancerous samples (unknown status)	18/55 (21/55)	0	10/20	0
Mean age (range)	unknown	unknown	72 (62–85)	43 (27–83)
Beta globin +	55/55	16/16	20/20	10/10
XMRV +	0	0	2/20	0
MuLV +	0	0	4/20	0
IAP +	0	0	5/20	0
mtDNA +	nd	nd	2/10	nd

(b) Specific PCR results from Indian samples

Indian sample number	Cancer status	PCR result using specific primers				
		β-globin	IAP	mtDNA	MLV *gag*	XMRV LTR
6489c/10	cancer	+	+	+	+	+
5383c/10	cancer	+	+	–	+	+
5406a3/10	cancer	+	+	–	–	–
2896c/10	BPH	+	+	+	+	–
5349c/10	cancer	+	+	–	+	–

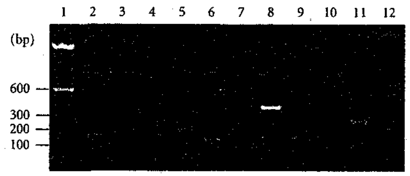

Figure 1: lane 1: MWM; lanes 2–4: β-globin PCR on LNCaP DNA template 1st round product, 2nd round product, and no-template control; lanes 5–7: XMRV LTR PCR on VP62 plasmid DNA template 1st round product, 2nd round product, and no-template control; lanes 8–10: MuLV *gag* PCR on VP62 plasmid DNA template 1st round product, 2nd round product, and no-template control; lanes 11-12: IAP PCR on McCoy cell DNA template and no-template control.

3.2. XMRV Detection in Whole Blood by Real-Time PCR. XMRV proviral DNA was not amplified from whole-blood extracts derived from 540 donors. The average DNA input for each amplification was 93,000 cells (approx 0.56 μg). Detection of XMRV/MuLV RNA was undertaken on a further 600 donors and 400 plasma minipools, derived from 19,200 individual donations. All samples tested negative for XMRV and MuLV sequences.

4. Discussion

Using highly sensitive PCRs with primers that detect XMRV and primers that detect MuLV-like sequences, no proviral DNA was detected in any of the prostate cancer samples independently of murine DNA contamination. This served to confirm our previous studies in which FFPE prostate tissue was tested and XMRV/MuLV sequences failed to be amplified [28]. Here we have added further data to show that no XMRV or MuLV-like sequences can be detected in fresh UK prostate tissue or in prostate cancer samples collected from Japan. Samples from India showed evidence of MuLV and XMRV sequences when viral genomic sequences were amplified by nested PCR. However, this was concordant with murine genomic DNA contamination detected using primers to IAP. IAPs are retrotransposons present at the level of around 1000 copies per mouse genome [30]. Thus, IAP PCR represents a highly sensitive detection method for murine DNA. Although the sample size was small ($n = 10$), we found no evidence to suggest that XMRV might be involved in other cancers, such as diffuse large B-cell lymphoma.

It was reported last year that XMRV had been detected in greater than 60% of 50 samples from English blood donors [36]. In contrast, we found no evidence of XMRV or pMuLV in any of 540 whole-blood samples from unselected NHSBT donors nor were we able to detect MuLV-like sequences in either the DNA from whole blood or cDNA prepared from the plasma minipools from donors in England. There are three possible explanations for this. Firstly, there are no MuLV infections in blood donors in England. Secondly, there are MuLV infections, but that the assays used failed to detect them, either due to sensitivity or sequence variation. Thirdly, there are MuLV infections, but the prevalence is too low to be detected in the sample sizes tested.

Research into the presence of MuLVs in the human population is contentious, given discrepant findings [37–39]. Contamination from sequences contained in apparently XMRV-positive samples, amplified products, or plasmids has been suggested as a reason for the finding of MuLVs in human samples [30, 40]. A study of XMRV in patients with CFS or chronic immunomodulatory conditions, using Invitrogen Platinum Taq (IPT), reported a *gag* sequence with >99% homology to a mouse endogenous retrovirus [19]. This was designated as contamination, although the paper failed to speculate on the source of this sequence. Sato and colleagues (2010) recently reported finding predominantly RNA sequences, related to a pMuLV, in IPT containing reagents [30]. Another study concluded that the detection of MuLV-related sequences in human samples could be due to contamination with mouse DNA, most likely contained in various laboratory reagents [29]. We have demonstrated that murine sequences can be present in prostate sections, resulting in false positive detection of XMRV [28]. A phylogenetic overview concluded that the proviral sequences present in the genome of 22Rv1 cell line were ancestral to the published XMRV sequences [31]; finally, it has been shown that the mapping of integration sites of XMRV in prostate cancer tissues, thought to unequivocally confirm the existence of XMRV in clinical samples, was at least partially contaminant derived [41], further emphasising the ease with which contamination can occur.

The sources of contamination are still to be fully elucidated. However, given that most retroviral laboratories have worked with MuLV or MuLV-derived vector systems, or at least used murine reagents, it is essential that sufficient appropriate controls are included in all PCRs.

The absence of MuLVs from all the samples analysed in this study, where there was no concomitant detection of murine genomic sequences, adds weight to the growing body of data questioning the evidence for murine retrovirus infection of humans [42]. It is always challenging to prove a negative result, but it is likely that XMRV will be added to the long list of RNA rumour viruses [43].

Conflict of Interests

The authors declare no conflict of interests.

Acknowledgments

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文献3

Research Article

Nucleic Acid, Antibody, and Virus Culture Methods to Detect Xenotropic MLV-Related Virus in Human Blood Samples

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The MLV-related retrovirus, XMRV, was recently identified and reported to be associated with both prostate cancer and chronic fatigue syndrome. At the National Cancer Institute-Frederick, MD (NCI-Frederick), we developed highly sensitive methods to detect XMRV nucleic acids, antibodies, and replication competent virus. Analysis of XMRV-spiked samples and/or specimens from two pigtail macaques experimentally inoculated with 22Rv1 cell-derived XMRV confirmed the ability of the assays used to detect XMRV RNA and DNA, and culture isolatable virus when present, along with XMRV reactive antibody responses. Using these assays, we did not detect evidence of XMRV in blood samples ($N = 134$) or prostate specimens ($N = 19$) from two independent cohorts of patients with prostate cancer. Previous studies detected XMRV in prostate tissues. In the present study, we primarily investigated the levels of XMRV in blood plasma samples collected from patients with prostate cancer. These results demonstrate that while XMRV-related assays developed at the NCI-Frederick can readily measure XMRV nucleic acids, antibodies, and replication competent virus, no evidence of XMRV was found in the blood of patients with prostate cancer.

1. Introduction

Xenotropic murine leukemia virus-related virus (XMRV) is a recently discovered gammaretrovirus reportedly associated with prostate cancer and chronic fatigue syndrome (CFS) [1, 2]. The discovery of XMRV arose from studies investigating a potential viral cause for diseases in patients with an *RNAseL* gene variant. This genotype, which is observed in a varying subset of patients in cohorts with prostate cancer [1, 3–8], has been associated with impairment of innate immune responses to viral infections [5]. Seeking an etiologically significant viral infection associated with impaired *RNAse L*-dependent responses, Urisman et al. first identified XMRV in 2006 in a cohort of prostate cancer patients [2]. The association of XMRV with prostate cancer, but not its association with the *RNAseL* variant, was corroborated by Schlaberg et al. in 2009 [9]. The prostate cancer studies were followed by a report from Lombardi et al. presenting evidence for XMRV infection in 67% of individuals with severe CFS, compared to 3.7% of healthy individuals [1]. These high reported frequencies of XMRV infection and putative linkage to a debilitating illness prompted concerns about the possibility of a new, widespread retroviral epidemic and stimulated additional research towards determining the prevalence of XMRV infection in different populations worldwide.

Several studies supporting high prevalence of XMRV infection followed. For example, Arnold et al. detected anti-XMRV antibodies in 27% of individuals with prostate cancer [10], Schlaberg et al. found XMRV nucleic acid in 23% of prostate cancers and 4% of controls [11], and Danielson et al. detected XMRV in 22.8% of extracted prostate tissues from individuals who had radical prostatectomies [12]. However, controversy arose when other laboratories could not demonstrate comparable findings in similar cohorts not only in the US [13] but in Germany [14], The Netherlands [15], and England [16, 17]. Adding to the controversy, Lo et al. reported the presence of mouse retroviral sequences, but not XMRV, in 86.5% of CFS patients [18]. Claims were made that such findings supported the association of XMRV infection with CFS, complicating an already controversial field.

Several factors were speculatively proposed to contribute to the differential detection of XMRV/MLVs by different laboratories. It was suggested that inconsistencies in detection of XMRV/MLVs in patient samples could result from varied prevalence of infection in different populations, differing criteria for patient selection, and differing detection methodologies utilized [19]. It was also proposed that virus levels may be chronically low or episodic in patient plasma or tissues, making virus detection difficult [19]. Adding to the complexity, detection of XMRV by PCR is highly susceptible to false positive results due to the very close genetic relationship of XMRV with endogenous MLVs and the high prevalence of contaminating mouse genomic DNA in many specimens [20, 21]. Indeed, studies have suggested that XMRV detection is the result of laboratory contamination from infected cell lines [22–25] or contaminated reagents [26]. Further suggestions of laboratory contamination came after publication of a study by Paprotka et al. [25], showing that XMRV originated in a human cancer cell line generated by passaging prostate cancer cells through immunocompromised mice. This result indicates that XMRV could not have entered the human population until recently, yet was already being reported as prevalent in a sizeable fraction of prostatic cancers. Furthermore, it showed that most "XMRV-specific" detection assays could, in fact, detect one or the other of the two parental proviruses (PreXMRV-1 and 2) that gave rise to XMRV and are endogenous to some inbred and wild mice. In assessing this situation, it became clear that to rule out false positive results and reliably detect XMRV infection, one must apply several diagnostic methods used in conjunction with known positive and negative controls.

At the NCI-Frederick, we sought to help clarify the XMRV controversy by generating multiple assays, including rigorous methods to measure antibodies to XMRV through ELISA-based methods, to quantify XMRV proviral DNA and viral RNA through quantitative PCR and RT-PCR methods, and to measure infectious virus by viral isolation cultures using an indicator cell line system. We characterized these assays using available positive and negative control samples, including spiked samples and specimens from two pigtail macaques experimentally inoculated with XMRV. We then applied these methods to specimens from two cohorts of prostate cancer patients to determine the levels of XMRV in their blood. Overall, we observed a high level of concordance between detection methods and were able to rule out false positive results by applying multiple assays on the same patient samples. Applying this approach, we did not find evidence of XMRV infection in any of the prostate cancer patient-derived specimens studied.

2. Methods

2.1. Clinical Prostate Cancer Samples. The XMRV detection assays developed at the NCI-Frederick were applied to samples collected from two cohorts of prostate cancer patients. In total, 134 patients were studied. Plasma samples from 108 patients were obtained at the UC Davis Cancer Center. Samples were collected between 2006 and 2010 from prostate cancer patients who were either newly diagnosed, on active treatment, or undergoing post-treatment monitoring. Plasma from all 108 patients was tested for XMRV RNA and antibodies to CA and TM. Institutional Review Board (IRB) approval was obtained from the UC Davis Cancer Center Biorepository, and all study subjects provided written informed consent.

Samples from an additional 26 recently diagnosed prostate cancer patients were obtained from the Urologic Oncology Branch, NIH Clinical Center, Bethesda, MD. All 26 blood samples were tested for the presence of XMRV RNA in plasma and DNA in whole blood. Tests for XMRV proviral DNA were also performed on prostate tissue from 19 of the 26 individuals in this cohort who had radical prostatectomies. Twenty-two of 26 blood samples were tested for antibodies to CA and TM. A subset of 12 samples was tested by virus rescue culture including those that had positive or indeterminate results by X-SCA or ELISA and matched negative controls. The study was approved by the IRB of NCI, NIH, Bethesda, MD, and all study subjects provided written informed consent.

2.2. XMRV Nucleic Assay Detection with XMRV Single-Copy Assays (X-SCA). Similar to the single-copy assay (SCA) for human immunodeficiency virus (HIV) [27], quantitative real-time PCR and RT-PCR assays for detection of XMRV, called XMRV single-copy assays (X-SCA), were developed to quantify XMRV nucleic acid in plasma, whole blood, and cell suspensions obtained from blood or tissue samples. The assays were designed using amplification primers targeting a *gag* leader region conserved between XMRV (as well as PreXMRV-2 [25]) and non-XMRV endogenous MLVs (forward 5-TGTATCAGTTAACCTACCCGAGT-3′, reverse 5-AGACGGGGGCGGGAAGTGTCTC-3′). Consequently, efficient amplification is achieved from both target templates allowing detection of either XMRV or MLVs present in patient samples. The Taqman probe (5′fam-TGG AGT GGC TTT GTT GGG GGA CGA- tamra3′) used for detection of amplified products was designed to span a signature 24 nucleotide deletion in the XMRV (PreXMRV-2) *gag* leader that differentiates these from all other MLV sequences (Figure 1(a)). In the event that a positive sample is identified by X-SCA, single-genome sequencing should be performed to confirm that the source of amplification was XMRV and

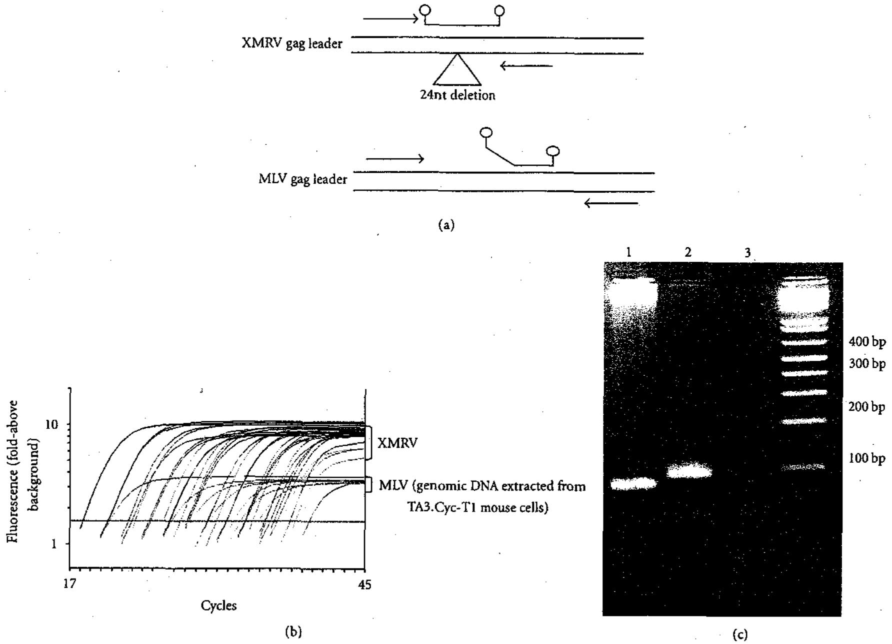


Figure 1: *XMRV single-copy assay (X-SCA). X-SCA primers anneal to conserved regions in XMRV/MLV gag leader region while the probe* spans a 24 nt deletion in XMRV compared to MLV (a) allowing for differential amplification profiles for XMRV and MLV (b). X-SCA amplification products run on a 2% agarose gel distinguish between the products being amplified since the XMRV product is 24 nt smaller than the MLV product. Lane 1 is the X-SCA product from the XMRV standard curve, Lane 2 is the MLV product from the genomic DNA extracted from TA3.Cyc-T1 mouse cells, and Lane 3 is the "no template" negative control (c).

not contaminating mouse DNA with a similar gag deletion, such as PreXMRV-2.

XMRV RNA was extracted from plasma samples following ultracentrifugation exactly as described for HIV SCA [27] and genomic DNA was extracted and whole blood samples using the Promega genomic DNA Extraction Kit (Cat no. A1120) according to the manufacturer's suggested protocol. Reaction conditions for synthesizing cDNA and measuring RNA copy number were exactly as described previously for HIV SCA [27]. XMRV proviral copy number was determined using the Lightcycler 480 Probes Master (Cat no. 04707494001) according to protocol and by performing 45 cycles of 95°C for 15 seconds, 60°C for 1 minute after an initial 10 minute, 95°C polymerase activation step. Accurate detection of XMRV by X-SCA was verified by testing spiked human blood products [28] and by testing blood samples collected from XMRV inoculated macaques (Del Prete et al., in preparation). Pigtail macaques were experimentally inoculated with XMRV (~4.8 × 10^9 RNA copy equivalents) prepared from the supernatant of 22Rv1 cells (Lot SP1592, Biological Products Core, AIDS and Cancer Virus Program, SAIC-Frederick, Inc, NCI-Frederick). Plasma and PBMC samples were collected prior to inoculation and through 119 days after inoculation. These pre- and post-inoculation specimens were used as reference control samples in evaluating X-SCA methods for detection of XMRV. Details of the macaque infection study will be reported elsewhere (Del Prete et al. in preparation). Animals were housed and cared for in accordance with American Association for Accreditation of Laboratory Animal Care (AALAC) standards in an AAALAC accredited facility, and all animal procedures were performed according to a protocol approved by the Institutional Animal Care and Use Committee of the National Cancer Institute. Detection of MLV was qualified by extracting mouse genomic DNA from TA3.Cyc-T1 cells using the Promega genomic DNA Extraction Kit (Cat no. A1120) and performing X-SCA in duplicate on dilutions of 3000 to 0.03 cell equivalents.

All patient samples were tested by X-SCA in duplicate or triplicate with equal numbers of no template controls (NTC) to monitor the level of false positives due to either viral or mouse genomic DNA contamination. The level of detection for XMRV nucleic acid in clinical samples was determined by the volume of sample available for testing (100 μL to 3 mL). Therefore, X-SCA sensitivity varied from 0.6 to 20.6 copies/mL of plasma and 0.9–10 copies/mL in whole blood. Because of the high frequency of false positives due to contaminating mouse DNA, we set strict criteria for declaring a sample positive for XMRV, requiring detection of viral sequence in all replicate PCR reactions from the samples being tested. These criteria result in a minimum detection of 1.8–41.2 copies XMRV RNA/mL in plasma and 2.7–30 copies XMRV DNA/mL in whole blood for a positive X-SCA test, depending on the volume of sample being tested. If discordant results are obtained from duplicate or triplicate wells, then the result is considered indeterminate and is repeated where sufficient sample is available.

2.3. XMRV Serology. XMRV antigens were prepared in the Protein Expression Laboratory, SAIC-Frederick, MD, as previously described [29]. Purified XMRV antigens were used to develop and optimize ELISA-based protocols (Bagni et al., in preparation). Briefly, purified CA and TM were spotted onto Meso Scale Discovery (MSD) (Gaithersburg, MD) standard 96-well plates at 8 μg/mL and 2 μg/mL, respectively. Samples were diluted 1 : 100 and incubated with individual XMRV antigens. Human antibodies were detected using biotin labeled anti-human IgG (Jackson ImmunoResearch, West Grove, Pa) and MSD-proprietary Sulfo-tagged streptavidin detection reagent and read on a SECTOR Imager 6000 (MSD) plate reader. The XMRV serology assays were qualified with samples obtained from XMRV-inoculated macaques (Del Prete et al., in preparation). Patient samples were considered reactive if the MSD electrochemiluminescent signal (ECL) was at least 50% relative to the ECL signal of the macaque positive control sera. Less reactive patient samples that were at least 2 standard deviations above the average negative human sample were considered indeterminate.

2.4. XMRV Culture Detection. The presence of replication-competent XMRV was determined in a virus rescue coculture assay using indicator cells designated DERSE (Detectors of Exogenous Retroviral Sequence Elements) and using expression of a GFP reporter as the readout. DERSE.LiGP cells are a subclone of LNCaP cells (gift from Dr. Francis Ruscetti, NCI) stably transfected with pBabe.iGFP-puro and screened for susceptibility to XMRV infection (Lee et al., in preparation). pBabe.iGFP-puro is an MLV proviral vector that encodes an intron-interrupted reporter GFP gene and is only expressed after mobilization by an infecting gammaretrovirus for a second round of infection of DERSE.LiGP cells. Similar MLV vectors that only express a reporter after being propagated in infection have been described previously using HEK293 cells [30]. The DERSE.LiGP assay will detect any MLV-related viruses that are capable of replicating in human prostate cancer cells. Virus replication can be detected by monitoring GFP-positive cells either by fluorescence microscopy or FACS analysis.

DERSE.LiGP indicator cells were maintained in Roswell Park Memorial Institute (RPMI) media 1640 (Invitrogen) supplemented with 15% fetal bovine serum (FBS) (Hyclone), 1x Pen/Strep/Glutamine (100 U/mL Penicillin, 0.1 mg/mL Streptomycin, and 0.292 mg/mL Glutamine, Invitrogen) and 1 μg/mL puromycin (Calbiochem). DERSE.LiGP cells were plated at 1 × 10^5 cells/well in a 24-well tissue culture plate one day before infection. As a positive control, 22Rv1 cell supernatants were diluted in RPMI media and added to cells the next day in the presence of 5 μg/mL of polybrene [31]. Culture medium was refreshed the following day by replacement or splitting cells at a 1 : 3 ratio depending on cell density. Although GFP can be detected in positive control samples within 3 days of infection, to maximize sensitivity for detection of low levels of virus, DERSE.LiGP cells exposed to clinical specimens were maintained in culture for at least two weeks and observed at intervals by fluorescence microscopy. After two weeks, cells were resuspended in a 2% paraformaldehyde (PFA) solution and GFP expression was measured by FACS (FACSCalibur, Becton Dickinson), indicative of a spreading infection. While DERSE.LiGP cells are relatively insensitive to heparin, plasma samples containing EDTA are toxic to the cultures. To mitigate toxicity, 200 μL of EDTA containing plasma samples were distributed into Eppendorf tubes in the presence of 7.5 mM $CaCl_2$ to neutralize the EDTA and 30 U/mL heparin salt to minimize sample clotting. Tubes were incubated for 4 hrs at 4°C to separate the plasma from residual clotting. Accurate detection of XMRV by virus culture was verified using a dilution series of supernatants from 22Rv1 cells and XMRV-spiked human plasma samples containing approximately 10^7 to 10 copies of XMRV RNA. Using XMRV-spiked samples, we noted a loss of detection sensitivity of three- to fivefold in EDTA containing plasma samples treated in the above manner. A recent report of XMRV inactivation by human complement may explain in part the loss of infectivity after addition of plasma [24]. Prostate cancer samples with indeterminate results by X-SCA or ELISA were matched with negative samples and tested blinded in the virus culture assay.

We required that samples test positive for XMRV nucleic acid (RNA or DNA) and by at least one other detect method (immunoassay or culture assay) to be declared positive for XMRV infection.

All reagents developed at the NCI-Frederick and described here are being made available to the extramural research community through the NIH AIDS Research and Reference Reagent Program or AIDS and Cancer Virus Program, SAIC-Frederick, Inc., National Cancer Institute, Frederick.

3. Results

3.1. Differentiating between XMRV and MLV with X-SCA Probe. The X-SCA probe used for detection of amplified products spans a signature 24 nucleotide deletion in the XMRV [1] and in the PreXMRV-2 [32] *gag* leader that differentiates these from all other MLV sequences (Figure 1(a)). Amplifications of XMRV from 22Rv1 DNA and MLV from mouse genomic DNA (extracted from TA3.CycT1 cells) show that the probe design results in a lower level of plateau fluorescence from non-XMRV MLV templates than from XMRV templates (Figure 1(b)), likely due to inefficient binding and/or degradation of the probe during MLV extension compared to XMRV extension. The result of the probe design is differential amplification profiles for XMRV and MLV, indicating which product is being detected in the assay and the proportions of each if both templates are detected. To confirm the result, the products were run on an agarose gel (Figure 1(c)). The XMRV X-SCA product is 86 nt long and the MLV product 110 nt, easily distinguishable on a 2% agarose gel.

3.2. *Qualifying XMRV Assay Detection Capabilities with Spiked Human Samples.*

Assays for detection of XMRV nucleic acid and replication-competent virus were established using XMRV-spiked samples as positive control specimens. To determine the accuracy and sensitivity of X-SCA methods to detect XMRV in human blood products, we tested a full panel of plasma and whole blood samples that were spiked or not spiked with XMRV derived from 22Rv1 cells. The panel was blinded as to which samples were XMRV positive and which were XMRV negative and were provided to us by the XMRV Scientific Research Working Group for testing by X-SCA [28]. Results from the blinded panel of spiked samples were described previously by Simmons et al. [28] and demonstrated that we detected XMRV RNA and proviral DNA using X-SCA with 100% accuracy. The level of sensitivity for detecting XMRV RNA in the spiked plasma panel was limited by the volume of sample tested for XMRV (270 μL) to 3.3 RNA copies/mL. The level of sensitivity for detecting XMRV proviral DNA was a single XMRV-infected 22Rv1 cell in whole blood samples. All unspiked samples were properly reported as negative for XMRV detection indicating a very low rate of false positivity.

The use of DERSE.L-iG-P cells to detect XMRV was verified using 22Rv1 culture supernatants and XMRV-spiked human plasma. Figure 2 shows the results from virus rescue experiments performed under the following conditions (i) 22Rv1 supernatant alone, (ii) 22Rv1 supernatant treated with $CaCl_2$ and heparin, (iii) 22Rv1 supernatant spiked into human plasma treated with $CaCl_2$ and heparin. DERSE.LiGP cells treated with EDTA-containing human plasma alone are not viable. Proportions of GFP-positive cells detected by FACS at day 4 and day 8 after infection are shown in Figures 2(a) and 2(b). DERSE.LiGP cells exposed to 0.01 μL of 22Rv1 supernatant were GFP-positive by microscopy within 4 days of infection (Figure 2) demonstrating the sensitivity of this assay for detection of replication competent XMRV. The sensitivity of this detection decreased 3–5-fold in the presence of EDTA-containing plasma samples treated as described above. This decrease could in part be due to the presence of human complement as has been recently reported [24]. Additional days of culture increased the number of GFP-positive cells exposed to virus in the presence or absence of plasma. For this reason, cultures infected with human specimens were carried out for a minimum of two weeks.

Table 1: X-SCA Results on XMRV-inoculated macaques.

Monkey ID	Days after inoculation	Plasma XMRV RNA copies/mL	Copies of XMRV DNA per 10^6 PBMCs
14232	0	<1.1	0
14232	5	534.11	197
14232	119	<1.1	23
8242	0	<1.1	0
8242	13	2153.56	2833
8242	119	<1.1	645

Table 2: Immunoassay of plasma from XMRV-inoculated macaques.

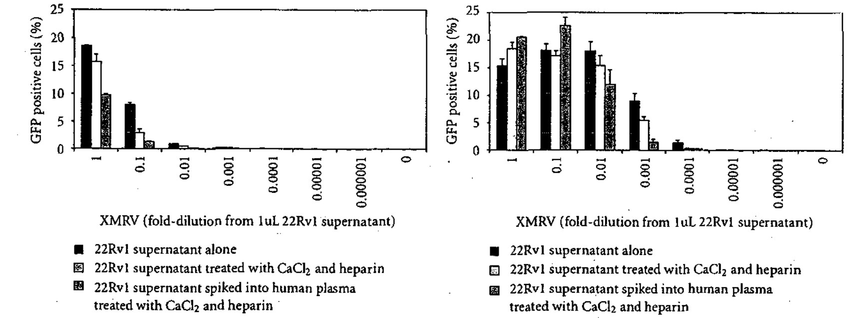
Monkey ID	Days after inoculation	Reactivity with	
		CA	TM
8242	0	19.5	248.5
8242	76	12713	544405
14232	0	14.5	145
14232	76	20108	285277

3.3. *Verifying Assay Detection Capabilities with Blood Samples from XMRV-Inoculated Macaques.*

To validate the specificity of X-SCA and ELISA, we used specimens from two pigtail macaques experimentally inoculated with XMRV. Detailed results from the macaque study will be reported elsewhere (Del Prete et al., in preparation). In short, samples tested by X-SCA revealed that peak viremia was achieved at 5 days after inoculation in one animal and at 13 days in the second (Table 1). By day 28, levels of XMRV RNA in plasma had declined to <1 copy/mL in both animals. PBMC-associated XMRV DNA was also measured by X-SCA. DNA levels peaked with similar kinetics as plasma viremia but persisted with levels of 23 and 645 copies/10^6 PBMC in the two animals, respectively, at the end of the follow-up period, 119 days after inoculation. Antibody reactivity to XMRV capsid (CA) and transmembrane protein (TM) measured by ELISA was undetectable prior to inoculation but were robustly positive thereafter (Table 2) (Del Prete et al., in preparation). Replication competent XMRV cannot be cultured from macaque plasma or PBMC samples due to extensive hypermutation of the provirus post-inoculation, likely due to the effect of APOBEC proteins (Del Prete et al., in preparation). Consequently, XMRV-spiked human plasma was used to verify the DERSE.L-iG-P cells for detection of XMRV.

3.4. *Testing Prostate Cancer Samples for XMRV Nucleic Acid, Antibodies, and Isolatable Virus.*

Samples obtained from the two cohorts of prostate cancer patients were assayed first for XMRV nucleic acid (X-SCA) and antibody reactivity against XMRV CA and TM protein (Tables 3 and 4). No plasma or prostate tissue samples in the NIH prostate cancer cohort or the UC Davis prostate cancer cohort were positive for XMRV nucleic acids or antibodies (Tables 3 and 4). However, two plasma samples in the NIH cohort (0594, 0771) were indeterminate for XMRV RNA. One of these samples (0594) was negative by ELISA, and the other (0771) had an indeterminate ELISA result. One other patient sample in the NIH cohort (0781) was indeterminate for XMRV antibody reactivity but negative for XMRV nucleic acid (Table 3). All three of these samples, along with 9 matched negative samples, were blinded and tested for replicating virus using the DERSE.L-iG-P assay. Virus could not be cultured from any of these plasma samples while it was readily recovered from positive control samples (22Rv1-derived XMRV spiked into negative human plasma) (Figure 3). Consequently, by our prospectively defined criteria, none of the 26 patient samples in the NIH cohort were considered to be XMRV infected (positive for nucleic acid, antibody, and/or replication competent virus) (Table 3). All 108 plasma samples from prostate cancer patients obtained from UC Davis were assayed for XMRV RNA and antibodies (Table 4). All samples were negative for XMRV nucleic acid except one (0739), which was indeterminate. No sample was found to be antibody reactive by our ELISA criteria (at least 50% reactive relative to the macaque positive control sera). Twelve of the 108 samples were indeterminate for XMRV reactivity to either CA or TM (2 standard deviations above the average negative human sample) but were negative for nucleic acid (Table 4). No sample was indeterminate or positive for both XMRV nucleic acid and antibody, and therefore, all were determined to be negative for XMRV infection.



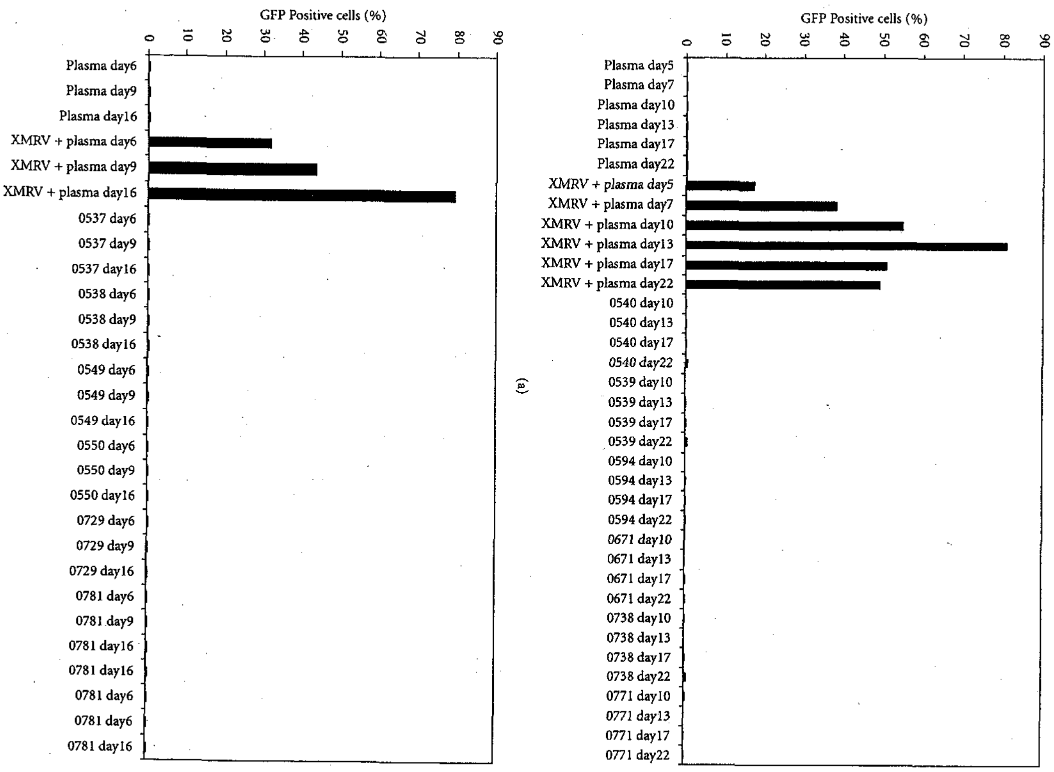
(a) (b)

Figure 2: *Verifying XMRV rescue by culturing on DERSE cells with 22Rv1 supernatants and with XMRV-spiked human plasma.* XMRV culturing under the following conditions: (i) 22Rv1 supernatant alone (black bars), (ii) 22Rv1 supernatant treated with $CaCl_2$ + heparin (white bars), (iii) 22Rv1 supernatant spiked into human plasma treated with $CaCl_2$ + heparin (gray bars). GFP-positive cells were analyzed by FACS at day 4 (a) and day 8 (b).

4. Discussion

After publication of the XMRV study by Lombardi et al. in October 2009 suggesting a possible disease association with CFS and a surprisingly high apparent seroprevalence for XMRV even among healthy control subjects, researchers at the NCI-Frederick set out to develop rigorous methods to evaluate the prevalence of XMRV infection. Using control samples, including spiked specimens where appropriate, we developed assays to measure plasma XMRV RNA viremia, cell-associated XMRV DNA levels, and antibodies to XMRV CA and TM. Because Lombardi et al. reported the presence of culture rescuable replication-competent virus from the blood of study subjects using coculture with a human cell line (LNCap), we created DERSE cells, derivatives of the same LNCap cells with a fluorescent reporter to detect XMRV replication. These cells broadly and sensitively detect the replication of different MLV-related gammaretroviruses that exhibit a tropism for human prostate cancer cells. In the absence of patient-derived definitive positive and negative control specimens, we applied our different assay methods to samples obtained from two pigtail macaques prior to and after experimental XMRV inoculation. XMRV plasma viremia was detectable in both inoculated macaques for 2-3 weeks after inoculation but then declined to undetectable levels (Del Prete et al., in preparation). However, XMRV DNA in PBMCs and serum antibodies remained at readily measurable levels for the duration of study follow-up in both animals (Del Prete et al., in preparation). Evaluation of samples from the inoculated macaques demonstrated the ability of our methods to reliably detect evidence of XMRV infection in blood samples and showed that XMRV provirus and antibodies persist even when viremia is not detectable.

In the development of diagnostic tools for XMRV infection, it became clear that a single method for XMRV detection would not be sufficient for definitive diagnosis due to a high frequency of false positives by PCR from contaminating nucleic acids (especially mouse genomic DNA) and high background reactivity seen by ELISA, even in samples



(a)

(b)

FIGURE 3: *Testing plasma samples from prostate cancer patients for replication competent XMRV.* Twelve samples were blinded as to their X-SCA and ELISA results and were tested for replicating virus using the DERSE.L-iG-P assay in two separate experiments. Six samples were tested in experiment 1 at passages 10, 13, 17, and 22 (a). All passages were negative for XMRV while virus was recovered from the positive control samples (10^7 copies of XMRV from 22Rv1 cells spiked into human plasma). Six additional samples were tested in experiment 2 at passages 6, 9, and 16 (b). All passages were negative for XMRV while virus was recovered from the positive control samples.

TABLE 3: X-SCA, ELISA, and virus culture results on prostate cancer samples from NIH cohort.

Sample number	Sample ID	Date of blood draw	Nucleic acid testing by X-SCA Plasma XMRV RNA copies/mL	XMRV DNA copies/mL in whole blood	XMRV DNA copies in prostate tissue	Number of prostate cells tested	Serologic Testing by ELISA CA	TM	ELISA result	Virus Culture *Virus* replication	Overall result
1	UB10-0533	8/5/2010	<0.6	<10	0	174000	999	985	NR	NT	NEGATIVE
2	UB10-0537	8/6/2010	<0.8	<10	NA	NA	5713	5302	NR	NEGATIVE	NEGATIVE
3	UB10-0538	8/6/2010	<0.7	<10	NA	NA	2323	2362	NR	NEGATIVE	NEGATIVE
4	UB10-0539	8/6/2010	<0.8	<0.9	NA	NA	1505	1864	NR	NEGATIVE	NEGATIVE
5	UB10-0540	8/6/2010	<0.8	<0.9	3.8	105600	1429	2811	NR	NEGATIVE	NEGATIVE
6	UB10-0542	8/9/2010	<0.7	<10	0	307500	1796	1949	NR	NT	NEGATIVE
7	UB10-0547	8/11/2010	<0.8	<10	0	81000	2248	4325	NR	NT	NEGATIVE
8	UB10-0548	8/11/2010	<0.8	<10	NA	NA	2566	2826	NR	NT	NEGATIVE
9	UB10-0549	8/11/2010	<0.8	<10	0	59025	5129	6059	NR	NEGATIVE	NEGATIVE
10	UB10-0550	8/11/2010	<0.8	<10	0	54825	1412	1414	NR	NEGATIVE	NEGATIVE
11	UB10-0578	8/19/2010	<0.8	<10	0	275250	1,412	1,460	NR	NT	NEGATIVE
12	UB10-0594	8/21/2010	*0.9*	<0.9	0	144075	3,050	3,190	NR	NEGATIVE	NEGATIVE
13	UB10-0643	9/14/2010	<0.7	<10	0	32400	5.359	5.430	NR	NT	NEGATIVE
14	UB10-0665	9/16/2010	<0.7	<10	0	84300	2.736	3.817	NR	NT	NEGATIVE
15	UB10-0671	9/21/2010	Invalid test	<0.9	NA	NA	1,490	1,375	NR	NEGATIVE	NEGATIVE
16	UB10-0706	9/28/2010	Invalid test	<10.0	0	3502.5	2.514	2.500	NR	NT	NEGATIVE
17	UB10-0729	9/30/2010	<0.7	<10.0	0	88950	1,453	1,620	NR	NEGATIVE	NEGATIVE
18	UB10-0771	10/14/2010	*10.4*	<0.9	NA	NA	10.655	26.030	*Equiv*	NEGATIVE	Indeterminate
19	UB10-0781	10/15/2010	<0.7	<10	NA	NA	8.151	8.451	*Equiv*	NEGATIVE	NEGATIVE
20	UB10-0738	10/5/2010	Invalid test	<0.9	0	74325	2.280	2.312	NR	NEGATIVE	NEGATIVE
21	UB10-0785	10/18/2010	<16.5	<10	0	27375	3.840	3.244	NR	NT	NEGATIVE
22	UB10-0788	10/19/2010	<16.5	<10	0	20887	2.990	2.596	NR	NT	NEGATIVE
23	UB10-0830	10/29/2010	<0.8	<10	0	63225	NT	NT	NT	NT	NEGATIVE
24	UB10-0833	11/1/2010	<0.8	<10	0	41175	NT	NT	NT	NT	NEGATIVE
25	UB10-0853	11/4/2010	<0.8	<10	0	8535	NT	NT	NT	NT	NEGATIVE
26	UB10-0913	11/19/2010	<0.8	<10	0	7132	NT	NT	NT	NT	NEGATIVE

NA: sample not available.
NT: sample not tested.

TABLE 4: X-SCA and ELISA results on prostate cancer samples from UC-Davis cohort.

	Plasma RNA				Plasma RNA		
Patient ID	Copies/mL	ELISA result	Overall result	Patient ID	Copies/mL	ELISA result	Overall result
P0005	<16.5	*Indeterminate*	NEGATIVE	P0566	<16.5	NR	NEGATIVE
P0013	<16.5	NR	NEGATIVE	P0572	<16.5	NR	NEGATIVE
P0015	<16.5	NR	NEGATIVE	P0592	<16.5	NR	NEGATIVE
P0024	<16.5	NR	NEGATIVE	P0593	<16.5	NR	NEGATIVE
P0026	<16.5	NR	NEGATIVE	P0605	<16.5	NR	NEGATIVE
P0027	<16.5	NR	NEGATIVE	P0611	<16.5	NR	NEGATIVE
P0031	<16.5	NR	NEGATIVE	P0612	<16.5	NR	NEGATIVE
P0034	<16.5	NR	NEGATIVE	P0617	<16.5	NR	NEGATIVE
P0036	<16.5	NR	NEGATIVE	P0632	<16.5	NR	NEGATIVE
P0044	<16.5	NR	NEGATIVE	P0637	<16.5	NR	NEGATIVE
P0045	<16.5	NR	NEGATIVE	P0641	<16.5	NR	NEGATIVE
P0118	<16.5	NR	NEGATIVE	P0650	<16.5	NR	NEGATIVE
P0133	<16.5	NR	NEGATIVE	P0657	<16.5	*Indeterminate*	NEGATIVE
P0144	<16.5	NR	NEGATIVE	P0659	<16.5	NR	NEGATIVE
P0154	<16.5	NR	NEGATIVE	P0672	<16.5	NR	NEGATIVE
P0156	<16.5	NR	NEGATIVE	P0673	<16.5	NR	NEGATIVE
P0162	<16.5	*Indeterminate*	NEGATIVE	P0675	<16.5	NR	NEGATIVE
P0167	<16.5	NR	NEGATIVE	P0679	<16.5	NR	NEGATIVE
P0170	<16.5	NR	NEGATIVE	P0685	<16.5	NR	NEGATIVE
P0172	<16.5	NR	NEGATIVE	P0710	<16.5	NR	NEGATIVE
P0177	<16.5	NR	NEGATIVE	P0721	<16.5	NR	NEGATIVE
P0185	<16.5	NR	NEGATIVE	P0723	<20.6	NR	NEGATIVE
P0195	<16.5	*Indeterminate*	NEGATIVE	P0726	<16.5	*Indeterminate*	NEGATIVE
P0209	<16.5	NR	NEGATIVE	P0733	<16.5	NR	NEGATIVE
P0219	<16.5	*Indeterminate*	NEGATIVE	P0739	*55*	NR	NEGATIVE
P0232	<16.5	NR	NEGATIVE	P0766	<20.6	NR	NEGATIVE
P0239	<16.5	NR	NEGATIVE	P0778	<16.5	NR	NEGATIVE
P0293	<16.5	NR	NEGATIVE	P0787	<16.5	NR	NEGATIVE
P0306	<16.5	NR	NEGATIVE	P0792	<16.5	NR	NEGATIVE
P0314	<16.5	NR	NEGATIVE	P0826	<16.5	NR	NEGATIVE
P0321	<16.5	NR	NEGATIVE	P0846	<16.5	NR	NEGATIVE
P0322	<16.5	NR	NEGATIVE	P0848	<16.5	NR	NEGATIVE
P0325	<16.5	NR	NEGATIVE	P0852	<20.6	NR	NEGATIVE
P0327	<16.5	NR	NEGATIVE	P0906	<16.5	*Indeterminate*	NEGATIVE
P0332	<16.5	*Indeterminate*	NEGATIVE	P0916	<16.5	NR	NEGATIVE
P0340	<16.5	*Indeterminate*	NEGATIVE	P0923	<16.5	NR	NEGATIVE
P0342	<16.5	NR	NEGATIVE	P0952	<16.5	NR	NEGATIVE
P0346	<16.5	*Indeterminate*	NEGATIVE	P0984	<16.5	NR	NEGATIVE
P0348	<16.5	NR	NEGATIVE	P0989	<16.5	NR	NEGATIVE
P0351	<16.5	NR	NEGATIVE	P0996	<16.5	NR	NEGATIVE
P0355	<16.5	NR	NEGATIVE	P0999	<16.5	NR	NEGATIVE
P0366	<20.6	NR	NEGATIVE	P1010	<16.5	NR	NEGATIVE
P0380	<16.5	NR	NEGATIVE	P1025	<16.5	NR	NEGATIVE
P0382	<16.5	NR	NEGATIVE	P1032	<16.5	NR	NEGATIVE
P0384	<16.5	NR	NEGATIVE	P1063	<16.5	NR	NEGATIVE
P0388	<16.5	NR	NEGATIVE	P1076	<16.5	NR	NEGATIVE
P0509	<16.5	*Indeterminate*	NEGATIVE	P1086	<16.5	NR	NEGATIVE
P0511	<16.5	NR	NEGATIVE	P1108	<16.5	*Indeterminate*	NEGATIVE
P0530	<16.5	NR	NEGATIVE	P1110	<16.5	NR	NEGATIVE
P0532	<16.5	NR	NEGATIVE	P1211	<16.5	NR	NEGATIVE
P0535	<16.5	NR	NEGATIVE	P1268	<16.5	NR	NEGATIVE
P0536	<16.5	NR	NEGATIVE	P1297	<16.5	NR	NEGATIVE
P0544	<16.5	NR	NEGATIVE	P1304	<16.5	NR	NEGATIVE
P0562	<16.5	NR	NEGATIVE	P1318	<16.5	NR	NEGATIVE

from healthy control subjects, presumably reflecting cross-reactivity. Therefore, we suggest a multiple assay approach to determine the XMRV status of patient samples. We established diagnostic criteria requiring that all replicates from X-SCA analysis must be positive and that serum antibodies and/or replicating virus must also be detectable in the same patient in order to report the patient XMRV positive. Samples resulting in discordant results from PCR replicates are reported as indeterminate. Despite earlier reports that evidence of XMRV infection was detected in as many as 20% of prostate tumors [2, 10–12], using the assays we developed, we did not find clear evidence for XMRV in the blood of two independent cohorts of patients with prostate cancer (total $n = 134$) or in the prostate tissue of a small subset of these individuals ($n = 19$). Based on previously reported frequencies of XMRV detection in prostate cancer patients, if XMRV is present in the blood of infected individuals, we expected that approximately 27 of the 134 patients in our study would be positive for XMRV. One patient from the NIH cohort (0771) had an indeterminate X-SCA result (2/3 reactions were positive for RNA). This sample was also positive for reactivity to CA and TM by ELISA. However, no XMRV DNA was found in the whole blood from this patient, and replication competent virus could not be recovered from the sample. Taken together, these data are considered an indeterminate result by our criteria. No other samples were positive by more than one diagnostic method.

The occasional positive X-SCA reaction is not above background for this assay. We regularly run 96-well plates of "no template controls" using both our X-SCA primers and primers targeting intracisternal A particles (IAP) [20, 21, 33] that are present in high copies in the mouse genome in order to monitor the levels of contaminating mouse DNA in the reagents and in the environment. We have found that about 5% of wells are positive with the X-SCA primers and about 20% with the IAP primers. Based on these backgrounds, we expect to detect low levels of mouse DNA contamination in samples tested, as seen is this study and in others [20, 21, 33]. Therefore, we required that all replicates of patient samples be positive to obtain a "positive" X-SCA result. We did not test the samples directly with IAP primers since we have not successfully found reagents and an environment that are free from mouse genomic DNA (on average about 1/3000 of a mouse genome per PCR reaction).

Although we had an occasional indeterminate result for XMRV RNA in the plasma samples studied, we did not detect XMRV DNA in any sample tested, despite the ability of our assay to sensitively detect XMRV DNA in spiked control samples and in specimens from inoculated macaques [28] (Del Prete et al., in preparation). Results from the inoculated macaques showed that in experimental infection, XMRV proviral DNA is readily measurable in blood cells even when plasma viremia was not detectable (Del Prete et al., in preparation), further suggesting that these patients do not carry XMRV in their blood. Findings from previous studies reporting higher prevalence for XMRV in similar cohorts [2, 11, 12] typically involved testing of prostate tumors. None of these studies reported the detection of XMRV in blood samples or the isolation of infectious virus from clinical specimens, and only one measured the presence of reactive antibodies through a virus neutralization assay [10]. Detection of antibody responses to specific viral proteins by ELISA or by reactivity to XMRV immunoblots was not assessed. If we had used less rigorous criteria basing an overall diagnosis on a single, nonconfirmed test and not requiring all replicates to yield the same result, then our two cohorts would have given rise to an apparent, and in our view almost certainly incorrect, reported XMRV prevalence rate of approximately 12%. These considerations may explain conflicting prior reports for the prevalence of XMRV and are consistent with claims that XMRV detection is likely the result of laboratory contamination [22, 26, 33, 34]. Particularly given the potential for false positive results in PCR and serological assays for XMRV, our results suggest that applying multiple diagnostic methods including measuring levels of proviral DNA in blood cells provides a more reliable approach for investigating the prevalence of XMRV. These results also demonstrate that XMRV nucleic acid, and antibodies are undetectable in the blood of patients with prostate cancer.

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Development and application of a high-throughput microneutralization assay: lack of xenotropic murine leukemia virus–related virus and/or murine leukemia virus detection in blood donors

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BACKGROUND: Xenotropic murine leukemia virus (MLV)-related virus (XMRV) and other related MLVs have been described with chronic fatigue syndrome and certain types of prostate cancer. In addition, prevalence rates as high as 7% have been reported in blood donors, raising the risk of transfusion-related transmission. Several laboratories have utilized microneutralization assays as a surrogate marker for detection of anti-MLV serologic responses—with up to 25% of prostate cancer patients reported to harbor neutralizing antibody responses.

STUDY DESIGN AND METHODS: We developed a high-throughput microneutralization assay for research studies on blood donors using retroviral vectors pseudotyped with XMRV-specific envelopes. Infection with these pseudotypes was neutralized by sera from both macaques and mice challenged with XMRV, but not preimmune serum. A total of 354 plasma samples from blood donors in the Reno/Tahoe area were screened for neutralization.

RESULTS: A total of 6.5% of donor samples gave moderate neutralization of XMRV, but not control pseudotypes. However, further testing by Western blot revealed no evidence of antibodies against MLVs in any of these samples. Furthermore, no evidence of infectious virus or viral nucleic acid was observed.

CONCLUSION: A microneutralization assay was developed for detection of XMRV and can be applied in a high-throughput format for large-scale studies. Although a proportion of blood donors demonstrated the ability to block XMRV envelope-mediated infection, we found no evidence that this inhibition was mediated by specific antibodies elicited by exposure to XMRV or MLV. It is likely that this moderate neutralization is mediated through another, nonspecific mechanism.

The short history of xenotropic murine leukemia virus (MLV)-related virus (XMRV) is one of controversy and discrepant results. Initial studies found XMRV nucleic acids and/or proteins in prostate cancers[1,2] and even a low percentage of prostate tissues from individuals with no history of prostate cancer.[2] In contrast, several other studies have failed to detect XMRV in prostate cancer tissue.[3,4] Much of this controversy is likely explained by polymerase chain reaction (PCR) and other nucleic acid contamination.[5,6] Despite XMRV originally being isolated from human prostate cancer samples,[1] it is in all likelihood a laboratory artifact, created by the passage of human prostate tissue through mice.[7] This resulted in infection with, and subsequent recombination between, at least two endogenous MLVs.[7] Cell lines created from this tissue, and harboring XMRV, were likely distributed to many laboratories working on prostate cancer.

ABBREVIATIONS: CFS = chronic fatigue syndrome; DEP = dual envelope pseudovirus; Lassa-GP = glycoprotein of Lassa virus; MLV = murine leukemia virus; MLV-P = polytropic MLV; qRT-PCR = quantitative reverse transcription–polymerase chain reaction; VSV-G = G protein of vesicular stomatitis virus; WB = Western blot; XMRV = xenotropic murine leukemia virus–related virus.

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The controversy surrounding the association between XMRV and chronic fatigue syndrome (CFS) is, if anything, greater. It was reported by Lombardi and colleagues[8] that two-thirds of CFS patients from the United States harbored XMRV compared to 4% of controls. Importantly, this work was based on three separate lines of evidence: 1) direct and indirect nucleic acid detection in peripheral blood mononuclear cells (PBMNCs), stimulated PBMNCs, and plasma; 2) culture of replication-competent XMRV from plasma and PBMNC by coculture with human prostate cells; and 3) serologic evidence using a flow cytometry assay. In addition to the association with CFS, the presence of virus in plasma and blood cells, coupled with the relatively high prevalence observed in apparently healthy controls, suggested that XMRV may be both blood transfusion transmitted and a real threat to the safety of the US blood supply. However, many other groups failed to detect XMRV in PBMNC samples from CFS or healthy individuals.[9-13] At least two studies tried to fully replicate the initial study using PCR, culture, and serology, without any convincing evidence of XMRV in either CFS patients or healthy controls.[14,15] Furthermore, more recent testing of specimens from the study by Lombardi and coworkers revealed that some of the previously reported PCR-positive specimens were contaminated with XMRV-containing plasmid sequences leading to the partial retraction of these PCR results from the publication by Lombardi and colleagues.[16] *Additionally, a recent* multilaboratory blinded study using 15 previously reported XMRV- or MLV-positive subjects as well as validated negative controls demonstrated that virus culture assays used in the study by Lombardi and colleagues were prone to cross-contamination.[17] Thus, this leaves only the serologic results as possible evidence for the presence of XMRV or other MLVs in humans. In the same multilaboratory study[15] the assays used by Lombardi and colleagues detected a serologic response in some specimens; however, this reactivity was not consistent within replicates of the same plasma sample and no statistical association was observed in CFS patients compared to blood donors, while three other highly sensitive assays in the study failed to detect a serologic response in any specimen.[17]

Microneutralization assays have been used extensively as diagnostic and specificity tests for many viruses, including alphaviruses and influenza.[18-20] Indeed, neutralizing antibodies are typically formed as part of a highly specific response to conformational epitopes. Neutralization of XMRV in 11 of 40 (27.5%) serum samples was observed in prostate cancer patients[21] suggesting that a microneutralization assay for XMRV would be feasible and useful. In this study, we generated a microneutralization assay for studies of blood donors seeking serologic evidence of XMRV or MLV infection based on the dual envelope pseudovirus (DEP) assay system we recently developed,[22] which has been proven to be a rapid, sensitive, and specific high-throughput system for antiviral drug discovery targeting viral entry. This assay system is composed of two viruses. Entry of the target virus is driven by the XMRV envelope protein pseudotyped onto the core of a reporter retrovirus, while infection by a second, internal control pseudovirus is mediated by an unrelated envelope and is included to reduce the number of false positives. Using this assay, we screened 354 donors and identified a small number with a neutralization signature warranting further testing.

MATERIALS AND METHODS

Sample collection

Anonymized plasma and whole blood aliquots were prepared using residual samples left over from pilot tubes collected for routine blood donation testing. The samples selected were from 354 different donations from the United Blood Services Reno facility. One or two ethylenediaminetetraacetate (EDTA) plasma tube(s) were used for preparation of these aliquots depending on the unit collection type. From each EDTA tube two plasma aliquots were prepared, the remaining sample was gently inverted to resuspend, and then three or four whole blood aliquots were prepared. All aliquots were frozen the day of preparation. Donor samples were coded to retain linkage only to the donor's zip code of residence, age, sex, and race/ethnicity. Any linkage to personal donor information such as name, address, and telephone number was removed. All samples provided were anonymized before shipment to Blood Systems Research Institute for subsequent testing. The institutional review board of the University of California San Francisco approved the study protocol.

Cells and reagents

Human embryonic kidney 293T cells clone 17 (293T/17) and human prostate LNCaP cells were obtained from the ATCC and grown in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum and penicillin and streptomycin (10 U/mL). LNCaP iGFP cells (DERSE, detectors of exogenous retroviral sequence elements) were provided by V. KewalRamani (NCI, Frederick, MD).

LacZ encoding polytropic MLV (MLV-P; termed LacZ[MCF13]) viruses were generated by first infecting NIH3T3 cells with replication-competent MCF13. The resulting cell line was then infected with replication-defective lacZ(A-MLV) pseudotype virus to introduce the lacZ gene.[23] CHO cells overexpressing murine ecotropic MLV receptor mCAT-1 (CERD9) have previously been described.[24,25]

Sera from wild mice experimentally or mock-infected with XMRV for 12 weeks[26] were used as positive and negative controls. XMRV-infected rhesus macaque (RII10 and RYH10) sera were provided by J. Hackett (Abbott, Abbott Park, IL).[27]

Plasmids

XMRV envelope (*env*) was PCR amplified from 22Rv1 cells with 100% nucleic acid sequence identity to the XMRV 22Rv1/CWR-R1 *env* sequence (GenBank Accession Number FN692043) and cloned into the pCAGGS vector with *KpnI* and *NheI* restriction sites. Plasmids encoding G protein of vesicular stomatitis virus (VSV-G), glycoprotein of Lassa virus (Lassa-GP), and the ecotropic MLV envelope have been described previously.[28-32]

Pseudotyped viruses with human immunodeficiency virus (HIV)-based retroviral backbone were generated from two plasmids, one encoding *env* and the other encoding the HIV backbone with a reporter gene. pNL4-3 Luc-R⁻E⁻ (pNL-luc) encodes a replication-incompetent variant of the HIV-1 molecular clone NL4-3, in which the *nef* gene has been replaced by a firefly luciferase (luc) reporter, and the *env* and *vpr* genes were inactivated, as previously described.[33] Similarly, pNL4-3 Ren-R⁻E⁻ (pNL-ren) was constructed by swapping the firefly luciferase gene for *Renilla* luciferase.[22] Pseudotyped viruses with MLV-based retroviral backbone were generated from three plasmids: XMRV *env*, MLV-based firefly luciferase reporter (MRP-luc),[34] and MLV gag/pol expression plasmid pHIT60.[35]

Virion production

HIV-based pseudovirions were produced essentially as previously described[30] by transfecting 293T/17 cells with 10 µg of the corresponding HIV construct (pNL-luc or pNL-ren vector) and 30 µg of plasmid encoding the viral envelope per 10-cm dish using the calcium phosphate transfection method. Similarly, MLV-based pseudovirions were produced by transfecting 5 µg of each of the three plasmid constructs per 10-cm dish. The next day, expression was induced with sodium butyrate (10 mmol/L) for 6 hours before washing the cells once with phosphate-buffered saline and then replacing the medium. Forty hours after transfection, the supernatant was filtered through a 0.45-µm pore size filter and frozen at −80°C. If required, virions were concentrated by ultracentrifuge concentration at 141,000 × *g* through a 20% sucrose cushion for 1.5 hours at 4°C. The pellets were resuspended in Hanks' buffered saline solution and aliquoted for storage at −80°C. Resulting reporter viruses were classified according to retroviral backbone, reporter system, and viral envelope, for example, MLV-luc(XMRV Env) or HIV-ren(Lassa-GP). LacZ encoding MLV-P was harvested from 3T3LacZMCF13 cells, filtered through a 0.45-µm pore size filter, and frozen at −80°C.

Microneutralization assay

Neutralization assays were performed in 96-well white tissue culture plates (Nunc, Rochester, NY). Donor serum samples were prepared from plasma by adding thrombin (King Pharmaceuticals, Bristol, TN) in 0.5 mol/L $MgCl_2$/$CaCl_2$ solution and then removing fibrin clots. The serum supernatant was transferred to a new tube and heat inactivated at 56°C for 30 minutes. A volume of 10 µL of serially diluted test sera or medium alone were transferred to assay wells, followed by 30 µL of either a single or a two-reporter virus mixture depending on the purpose of the assay and incubated for 1 hour at room temperature before addition of 40 µL of 293T/17 or LNCaP cells (500,000 cells/mL) to all wells. Plates were incubated for 2 days at 37°C and 5% CO_2 and firefly and *Renilla* luciferase reporter expression was determined sequentially as described in Zhou et al.[22] For the initial high-throughput microneutralization assays, sera samples with final dilutions of 80- and 240-fold were tested and each experiment repeated twice.

Neutralization dose response

For generation of neutralization dose-response curves with selected donor sera, samples were serially diluted starting from 40- or 80-fold initial dilutions. Assays were performed in triplicate. Infection of pseudoviruses MLV-luc(XMRV Env) and MLV-luc(VSV G) in 293T/17 cells and infection of MLV-luc(MLV-E Env) and MLV-luc(VSV G) in CERD9 cells were detected using a luciferase assay system (Bright-Glo, Promega, Madison, WI). Infection of LacZ encoding MLV-P in 293T/17 cells was detected using a system for chemiluminescent reporter detection of β-galactosidase (Galacto-Light Plus, Applied Biosystems, Foster City, CA). Additionally, the percentage of cells infected with LacZ encoding MLV-P was measured with cell fixation and visualization of blue color development under a microscope using a β-gal staining kit (Invitrogen).

Western blot

Western blot (WB) analysis was performed to detect XMRV or MLV antibodies in selected donor sera and healthy controls as previously described.[12,36] Briefly, XMRV-infected DU145 prostate cells (C7) were grown in complete HuMEC serum-free medium supplemented with 1% HuMEC and 50 µg/mL bovine pituitary extract (Invitrogen). Tissue culture supernatants were clarified by centrifugation and by passage through a 0.45-µm filter. XMRV was purified from 150 mL of C7 supernatant using a retrovirus maxiprep kit (ViraTrap, Bioland Scientific LLC, Paramount, CA) following the manufacturer's protocol. A volume of 150 µL of purified XMRV was denatured with sodium dodecyl sulfate–polyacrylamide gel electrophoresis sample buffer at 95°C for 10 minutes and viral proteins were separated by gel electrophoresis in a NuPAGE 4%-12% Bis-Tris gel (Invitrogen) for WB testing as previously described but modified by using horseradish peroxidase–conjugated protein G instead of protein A/G.[12,36] Seroreactivity was defined by reactivity to viral envelope and/or gag proteins of the expected size as seen in the positive control antisera. This WB test accurately detected XMRV antibodies in three experimentally infected macaques equivalent to detection using recombinant proteins in recently described immunoassays.[27]

Quantitative reverse transcription–PCR

RNA was extracted from 100 µL of selected donor whole blood samples using Qiagen Viral RNA Mini kit. The isolated RNA was subjected to reverse transcription by MLV reverse transcriptase (RT; Roche, Indianapolis, IN). The resulting cDNA was amplified in a real-time PCR procedure and quantified in a commercially available system (LightCycler 480, Roche). Quantitative reverse transcription (qRT)-PCR was performed with FastStart *Taq* polymerase (Roche) in 45 amplification cycles of 95 and 60°C for 30 seconds each. Two primer pairs were used, integrase (F2 [5′-AACCTGATGGCAGATCAAGC-3′], R2 [5′-CCCAGTTCCCGTAGTCTTTTGAG-3′], and XMRV probe [5′-FAM-AGTTCTAGAAACCTCTACACTC-BHQ1-3′])[13] or *gag* (Q445F [5′-GGACTTTTTGGAGTGGCTTTGTT-3′], Q528R [5′-GCGTAAAACCGAAAGCAAAAAT-3′], and XMRV probe F480PRO-BHQ [5′-FAM-ACAGAGACACTTCCCGCCCCCG-BHQ1-3′]).[37] A cutoff of 40 CTs was used as evidence for the presence of XMRV or MLV sequences in a specimen. Positive controls represented recombinant plasmid spiked into whole blood samples in a dilution series from 10^6 to 10^4 copies/mL.

Nested RT-PCR amplification of XMRV sequences

Nested RT-PCR was performed as described.[38] Briefly, RNA was extracted from 0.5 mL of donor plasma using a virus kit (QIAamp Ultrasens, Qiagen) and subjected to reverse transcription employing a first-strand synthesis system for RT-PCR (Superscript III, Invitrogen). Culture supernatant of the XMRV-producing prostate cancer cell line 22Rv1 was used at a 10^{-5} dilution as a positive control for RNA isolation. For amplification of XMRV *gag* sequences, 5 µL of the transcribed cDNA was used for the first round of 40-cycle amplification with primers 419F (5′-ATCAGTTAACCTACCCGAGTCGGAC-3′) and 1154R (5′-GCCGCCTCTTCTTCATTGTTCTC-3′)[8] and master mix (HotStart-IT FideliTaq, USB Corp., Cleveland, OH). Nested PCR was performed for 45-cycle amplification with 5 µL of the first-round PCR product and two different primer pairs, Gag-I-F (5′-TCTCGAGATCATGGGACAGA-3′) and Gag-I-R (5′-AGAGGGTAAGGGCAGGGTAA-3′) or NP116 (5′-CATGGGACAGACCGTAACTACC-3′) and NP117 (5′-GCAGATCGGGACGGAGGTTG-3′).[39] To monitor assay sensitivity, plasmid DNA containing a cloned fragment of XMRV *gag*[12] was included in each PCR run at concentrations from 1 to 100 copies/µL. PCR and RT-PCR of GAPDH controls with primer pairs, forward (5′-CATGTTCCAATATGATTCAC-3′) and reverse (5′-CCTGGAAGATGGTGATG-3′), were performed to ensure similar levels of DNA and RNA input in each round of amplification.

Propagation of infectious XMRV in indicator cells

DERSE (detectors of exogenous retroviral sequence elements) indicator cells were developed at the National Cancer Institute by stable transfection of pBabe.iGFP-puro into LNCaP cells. The intron interrupted GFP gene from pBabe.iGFP-puro is only expressed after mobilization by an infecting gammaretrovirus for a second round of infection.[40] To test for the presence of infectious XMRV in selected donor plasma, DERSE.Li-G cells were inoculated with donor plasma or control plasma and spin infection, as described in Steffen and colleagues.[38] GFP expression was monitored every 3 to 4 days for a total period of 3 weeks. As a positive control, culture supernatant of the XMRV-producing prostate cancer cell line 22Rv1 (containing roughly 10^9 copies/mL) was used as an inoculum at 10^{-2}, 10^{-4}, and 10^{-6} dilution.

RESULTS

High-throughput microneutralization assay development

XMRV pseudoviruses (MLV-luc[XMRV Env]) were generated using a MLV-based retroviral backbone. These pseudoviruses infected both 293T and LNCaP cells. As expected from previous studies,[41] levels of infection mediated by the XMRV envelope were somewhat lower compared to control envelopes. For example, on 293T/17 cells, infection of unconcentrated MLV-luc(XMRV Env) was about equal to VSV-G pseudotyped virus stocks diluted 10-fold (67,714 and 63,742 relative light units, respectively). On both cell types MLV-luc(XMRV Env) was neutralized by sera from mice (Fig. 1A) and rhesus macaques

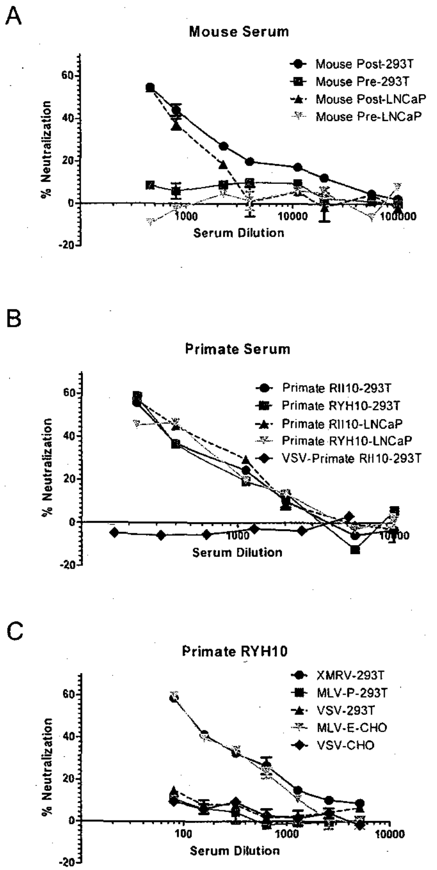

Fig. 1. Detection of XMRV Env neutralizing antibodies in positive controls. MLV-luc(XMRV Env) pseudovirus infection of 293T/17 and prostate LNCaP cells was neutralized by sera from both mice (A) and rhesus macaques (B) challenged with XMRV, whereas no clear neutralization was observed with preimmune sera or HIV-luc(VSV G) pseudoviruses. (C) MLV-luc(MLV-E Env) pseudoviruses were neutralized by sera from rhesus macaques challenged with XMRV in mCAT-1 expressing CHO cells (CERD9 cells), but no clear neutralization of LacZ encoding MLV-P or HIV-luc(VSV-G) pseudoviruses was observed in 293T/17 cells. Infection of pseudoviruses with firefly luciferase reporter was detected with a luciferase assay system (Bright-Glo, Promega), whereas infection of LacZ encoding MLV-P was measured using a system for detection of β-galactosidase (Galacto-Light Plus, Applied Biosystems). Absolute values for the no sera controls were as follows: MLV-luc(XMRV Env) gave 55,810 relative light units (RLU) on 293T cells and 20,213 RLU on LNCaP cells; HIV-luc(VSV-G) gave 65,961 RLU on 293T cells and 51,677 RLU on CHO cells; and MLV-P gave 32,356 RLU on 293T cells and MLV-luc(MLV-E Env) gave 41,771 RLU on CHO cells. Results are presented as percentage of neutralization and shown as mean ± SD of triplicate measurements. A representative experiment of at least two experiments is shown.

(Fig. 1B) challenged with XMRV, whereas no clear neutralization was observed with preimmune sera. Similar results were obtained with HIV-luc(XMRV Env) (data not shown). Moreover, ecotropic MLV pseudoviruses (MLV-luc[MLV-E Env]) were also neutralized by sera from rhesus macaques challenged with XMRV (Fig. 1C). However, LacZ encoding MLV-P or HIV-luc(VSV G) pseudoviruses (Figs. 1B and 1C) were not neutralized.

To develop a reliable high-throughput assay system for the screening of large numbers of samples for XMRV infection, we generated a cell-based XMRV microneutralization assay system based on the internally controlled DEP assay we recently developed to screen for small-molecule inhibitors,[22] which has been proven to be a rapid, safe, sensitive, and specific high-throughput system for antiviral drug discovery targeting viral entry. We adopted a similar approach here for the XMRV microneutralization assay. The assays were performed in 96-well plate format with the aid of liquid dispensing equipment for high-throughput applications. After preliminary experiments, a combination of MLV-luc(XMRV Env) and HIV-ren(Lassa-GP), which showed no clear interference between the two envelopes, was chosen for the sera screening. This combination proved to give very robust and reproducible results. A combination of MLV-luc (XMRV Env) and HIV-ren(Lassa-GP) from three 96-well plates indicated that the interplate coefficient of variation (CV)[42] was 8.2 and 5.2% for MLV-luc(XMRV Env) and HIV-ren(Lassa-GP), respectively. A set of 20 sera samples indicated that for the intraassays, the CV of every sample in triplicate was within 5% and for the interassays, the CV of every sample from three plates was within 12%, for both MLV-luc(XMRV Env) and HIV-ren(Lassa-GP) (data not shown).

Generally, sera showed relatively higher levels of neutralization of XMRV Env pseudoviruses (approx. 30%) than the Lassa-GP control (approx. 8%; Fig. 2). Similar results were obtained with sera at 240-fold dilutions, in individual virus alone, and in LNCaP cells (data not shown). Despite this higher level of background neutralization, neutralization with a number of sera was noticeably more pronounced. For example, in Fig. 2, three (B37, B58, and B80) *of more than 80 donor sera* showed approximately 50% reduction in XMRV Env, but not Lassa-GP, mediated viral infection in 293T/17 cells.

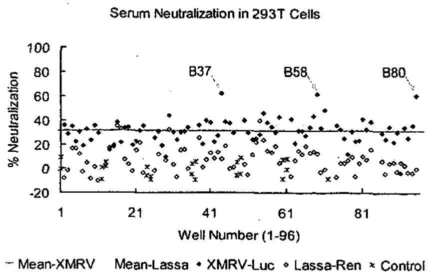

Fig. 2. XMRV Env neutralizing antibody in blood donor sera using a cell-based XMRV microneutralization assay system. Shown is an example screen of 80 donor serum samples (80-fold dilutions) for XMRV neutralization with virus combinations of MLV-luc(XMRV Env) and HIV-ren(Lassa-GP) *in a 96-well plate format. Three (B37, B58, and B80) of a total* of more than 80 donor sera showed approximately 50% reduction in XMRV Env-, but not Lassa-GP-, mediated viral infection in 293T/17 cells.

Screening of blood donors

We used this assay to screen a total of 354 blood donor sera collected within the United Blood Service region of Reno/Lake Tahoe. The Reno facility was chosen due to the collection territory including regions of Northern Nevada and California known to have clusters of CFS.[43-45] Patients *from CFS clusters, including the Reno/Lake Tahoe area,* formed the majority of subjects in the original demonstration of the presence of XMRV in blood.[8] Twenty-three sera gave more than 50% reduction in XMRV Env, but not Lassa-GP, mediated viral infection at either 80- or 240-fold dilutions. All 23 serum samples showed a dose-dependent neutralization of XMRV pseudoviruses (approx. 60% neutralization at 80-fold dilution), but unlike the mouse and macaque antisera, the blood donor sera demonstrated very limited neutralization for MLV-E pseudoviruses (<50% at 80-fold dilution; Fig. 3). No clear neutralization *was detected for VSV pseudoviruses (Fig. 3) and LacZ* encoding MLV-P (data not shown).

Confirmatory testing

The 23 moderately neutralizing sera (>50%) as well as 14 additional poor neutralizers (approx. 30%-50%) and 12 donors with no clear neutralizing ability (<30%) were further assessed with a recently developed WB assay[12,36] using purified, denatured XMRV antigen from XMRV-infected DU145 prostate cells (C7). All 50 of the tested blood donor sera were WB-negative (Fig. 4).

To further confirm whether there was any evidence of XMRV or other MLV infection in these individuals that would lead to a positive serologic response, we performed PCR assays and virus cultures that would detect both specifically XMRV and more broadly other MLVs. Whole blood samples of the selected donors were tested by qRT-PCR using primer sets located in either XMRV integrase[40] or *gag*. No positive signal was seen in any sample with either primer set (data not shown). Plasma samples of the 23 selected donors were also tested and found negative by nested RT-PCR using generic MLV primers previously shown to detect both XMRV and the broader family of xenotropic and polytropic MLVs[39] (Fig. 5).

To test for the presence of infectious MLVs in donor plasma, the indicator cell line DERSE was used. As a positive control, culture supernatant of the XMRV-producing prostate cancer cell line 22Rv1 (containing roughly RNA 10^9 copies/mL) was utilized as an inoculum. Whereas cells inoculated with 22Rv1 supernatants showed a concentration-dependent GFP expression on Day 7 and spread of the virus on Day 21 as previously described,[38] no GFP expression could be observed in any of the cells inoculated with donor plasma from the 23 seroreactive persons, even when spin infection was used to enhance the potential infection efficiency (data not shown).

DISCUSSION

Determining whether serologic evidence of immune responses to gammaretroviruses in humans[8,21] is an indication of authentic infection or just nonspecific cross-reactivity is an important final step in the XMRV saga. In this study, we generated a robust, high-throughput microneutralization assay for the screening of large numbers of subjects for serologic evidence of XMRV and MLV infection based on the DEP assay system we recently developed.[22] This assay includes an internal control pseudovirus that is very useful for avoiding nonspecific inhibition and also controls for cytotoxicity. This method provides a reproducible high-throughput microneutralization research assay for large-scale testing for evidence of XMRV and MLV infection.

Currently, enzyme immunoassays (EIAs) and WB are the two most common serologic methods utilized for viral diagnosis.[46,47] WB is limited to the recognition of linear epitopes and is prone to high-background rates, while EIA can be restricted by the quality of the antigens, antibodies, and detection methods. Instead of directly detecting the existence of antiviral antibodies in the sera, the DEP-based microneutralization assay is based on the ability of a serum to neutralize pseudovirus infection. Compared with standard assays such as EIAs, the microneutraliza-

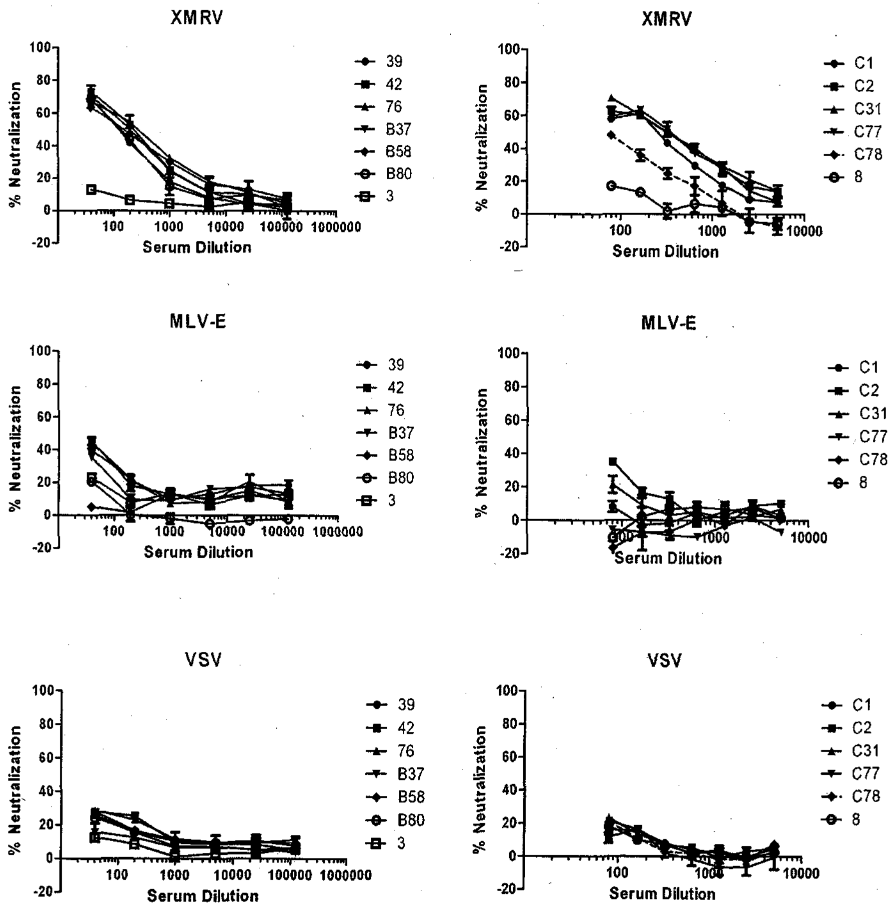

Fig. 3. Dose-response curves with selected blood donor sera. Neutralization of infection of HIV-luc(XMRV Env) and HIV-luc(VSV G) pseudoviruses with serially diluted donor sera samples were detected in 293T/17 cells and HIV-luc(MLV-E Env) in mCAT-1 expressed CHO cells (CERD9 cell). Results are presented as percentage of neutralization and shown as mean ± SD of triplicate measurements. A representative of at least two experiments is shown.

tion assay has fewer steps and can be performed by automated liquid handling equipment, which may generate less SD. The disadvantage is a 2-day incubation period which impacts the clinical usefulness of the assay.

A recent study identified neutralizing activity against XMRV in approximately 14% of blood donor samples,[10] although in this instance many of these sera neutralized control viruses in addition to XMRV. In contrast, while we identified 23 of 354 blood donors (6.5%) able to moderately neutralize XMRV Env-mediated infection, control and other MLV envelopes were poorly or not at all neutralized. None of the samples tested showed any evidence of a serologic response to XMRV by WB testing. Furthermore, all 23 seroreactive samples were negative for XMRV and MLV sequences using PCR or virus culture. These PCR and culture assays were designed to detect a broad range of gammaretroviruses, as well as XMRV specifically, thus excluding XMRV/MLV and other gammaretroviruses as a source of the nonspecific reactivity. The finding that neutralization by the 23 blood donors was specific to XMRV envelopes, but not other MLV envelopes, was surprising. Pairwise comparison of the amino acid sequence of the envelope region between XMRV and MLV-P or MLV-E shows the amino acid similarity is approximately 89 and 68%, respectively.

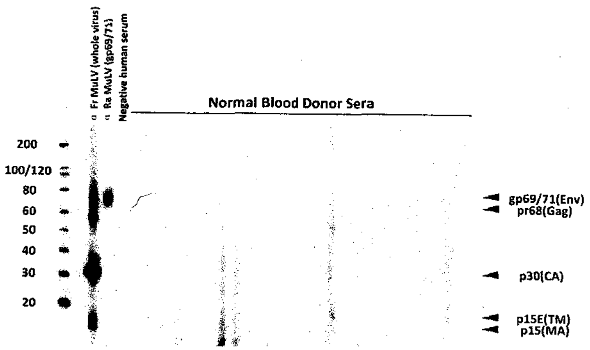

Fig. 4. Absence of XMRV/MLV antibodies in blood donor sera by WB analysis. Purified, denatured XMRV antigen from XMRV-infected DU145 prostate cells (C7) was used for WB detection of XMRV or MLV antibodies in selected donor sera samples. Results of positive control antisera to purified XMRV antigen and 24 normal donor sera samples (B58, B80, E6, E8, E10, D17, D40, C5, C20, C30, C33, C35, C45, C47, C49, C50, C51, C67, 3, 4, 5, 6, 7, 8, from left to right) are shown; locations of reactivity to specific viral proteins are indicated. Env (gp69/71) = envelope; TM (p15E) = transmembrane; Gag (pr68); MA (p15) = matrix; CA (p30) = capsid. Molecular weight markers (kDa) are provided on the left of the WB.

Given that the true XMRV neutralizing responses raised in animals were more broadly neutralizing (Fig. 1), this result strongly argues against specific neutralization, but rather suggests the moderate neutralization observed was mediated by other nonspecific means. This could be cross-reactive antibodies raised against endogenous retroviral elements, completely unrelated proteins, or other nonantibody serum factors. Human serum potently inhibits

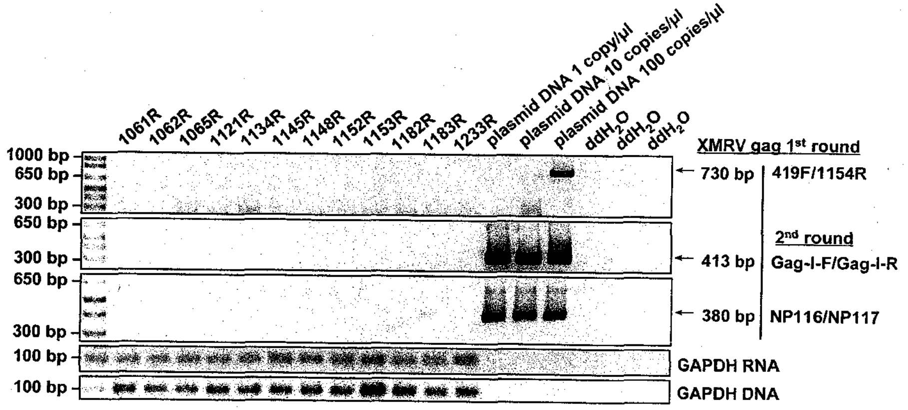

Fig. 5. Absence of XMRV gag sequences in blood donor plasma by nested RT-PCR. A representative result of 12 donor samples is shown with positive controls containing 1 to 100 copies/μL of a plasmid harboring a cloned fragment of XMRV gag[12] and negative water controls. First-round PCR amplification used primer pair 419F and 1154R and second-round PCR amplification used primer pairs Gag-I-F and Gag-I-R or NP116 and NP117. GAPDH RNA and DNA PCR results for the same samples are shown in the bottom two panels.

XMRV;[14] however, this is largely complement driven, and in our assay serum complement was inactivated by heating and did not influence our test results. The relatively high level of nonspecificity is greater than that seen with other microneutralization assays[20,48] and is partly due to the lack of known human positive cases that can be used to accurately set cutoffs for defining specific neutralization. Our results likely also explain other reported XMRV neutralization results in human samples.[21]

In addition to the initial association of XMRV to CFS made by Lombardi and colleagues,[8] a second publication by Lo and colleagues,[39] based only on PCR analysis, also yielded a strong association between CFS and MLV-like viruses.[39] These subsequent viruses demonstrated a far greater degree of sequence variation than XMRV, with the majority of sequences resembling P-MLV. Although Lo and coworkers[39] reported very stringent measures to minimize contamination, the most parsimonious explanation, given the extent of reported contamination of laboratory reagents, is that their PCR results are false positives resulting from reagent contamination. Indeed, Lo and colleagues used platinum *Taq* (Invitrogen) for PCR amplification, which several groups have convincingly demonstrated is contaminated with mouse DNA[14,15,49] due to the use of a mouse monoclonal antibody in the enzyme mix. Furthermore, recent detailed phylogenetic analysis of the longitudinal MLV-P sequences reported by Lo and coworkers showed that these sequences are inconsistent with retroviral evolution.[50] Nonetheless, the findings of Lo and colleagues raised the hypothesis that while XMRV itself is clearly a laboratory contaminant, the serologic responses detected in Lombardi and coworkers may be due to infection by other MLVs or gammaretroviruses. The serologic assay used by Lombardi and coworkers relies on antibody binding to the MLV spleen focus-forming virus (SFFV) Env expressed on the surface of cells. The logic of this assay is that conformationally dependent cross-reactive epitopes shared between this mouse gammaretrovirus and XMRV would bind XMRV antibodies, which would then be detected in a flow cytometry–based assay. However, it is likely that, as with our microneutralization assay, mammalian cell culture–based expression of an unrelated retrovirus Env would be highly prone to nonspecific cross-reactivity that can confound the testing and which requires clarification by WB analysis using purified antigen. Indeed, when the Lombardi and colleagues' flow-based assay was used by two laboratories on plasma specimens in a blinded study, high levels of nonspecific reactivity were observed.[15]

In conclusion, we developed a robust, high-throughput microneutralization assay to conduct studies seeking evidence of infection with XMRV and MLV. Although a small proportion of blood donors demonstrated the ability to block XMRV-mediated infection, we found no evidence that this inhibition was mediated by specific antibodies elicited by exposure to XMRV or related MLVs. It is likely that this moderate neutralization is mediated through another, nonspecific mechanism. Our findings also explain further the highly nonreproducible and nonspecific serologic responses detected with other assays.[8,17] In addition, this microneutralization assay system can be easily adapted to screen donor samples against other viruses with careful selection of matching partner virus envelopes, which will provide important information for neutralizing antibody responses and infectious disease profiles.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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文献5

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PLoS one

No Evidence for XMRV Nucleic Acids, Infectious Virus or Anti-XMRV Antibodies in Canadian Patients with Chronic Fatigue Syndrome

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Abstract

The gammaretroviruses xenotropic murine leukemia virus (MLV)-related virus (XMRV) and MLV have been reported to be more prevalent in plasma and peripheral blood mononuclear cells of chronic fatigue syndrome (CFS) patients than in healthy controls. Here, we report the complex analysis of whole blood and plasma samples from 58 CFS patients and 57 controls from Canada for the presence of XMRV/MLV nucleic acids, infectious virus, and XMRV/MLV-specific antibodies. Multiple techniques were employed, including nested and qRT-PCR, cell culture, and immunoblotting. We found no evidence of XMRV or MLV in humans and conclude that CFS is not associated with these gammaretroviruses.

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Introduction

Chronic fatigue syndrome (CFS), also commonly referred to as myalgic encephalomyelitis (ME), is a complex disorder with an unknown etiology which is characterized by disabling physical and mental fatigue and pain that lasts for at least 6 months and lacks any obvious cause [1,2]. The sudden onset of symptoms and underlying activation of inflammatory pathways suggest an infectious agent as the triggering factor. Numerous viral and non-viral pathogens have been investigated in the context of CFS with as yet inconclusive results [1,2]. The xenotropic murine leukemia virus (MLV)-related virus (XMRV) was initially identified in human prostate cancer cells in 2006 [3]. It has since been thought to be the only member of the gammaretrovirus family known to infect humans and its possible role in the development of prostate cancer has been widely discussed [4]. In 2009, Lombardi *et al.* reported the detection of XMRV in both peripheral blood mononuclear cells (PBMC) and plasma of 67% of a CFS patient cohort compared to 3.7% in healthy controls [5]. This study has gained a high level of attention and was thought to mark a possible break-through in CFS research. Several studies have since addressed the possible connection between XMRV infection and CFS or prostate cancer, and the resulting evidence is controversially discussed in the field [4]. While one study reported the presence of other MLV-like sequences in CFS patients [6], others identified mouse DNA, human cell lines or commercial laboratory reagents to be a possible source of MLV contamination [7]. Attempts to reproduce the initial findings in different CFS patient groups world-wide and in parts of the initial cohort have since failed [4,8,9]. Thus, more research is needed to resolve an association of MLV-like viruses in humans. In this study we performed an extensive analysis of whole blood and plasma samples from two well-characterized Canadian CFS patient cohorts and healthy controls utilizing multiple laboratory techniques, including nested and qRT-PCR, cell culture, and immunoblotting for the detection of XMRV/MLV nucleic acids, infectious virus, and XMRV/MLV-specific antibodies.

Materials and Methods

Ethics statement

All study protocols were reviewed and approved by the Human Research Ethics Boards of the University of Calgary and the University of Alberta and all study participants provided written informed consent. Laboratory testing of the samples was performed anonymously and blinded.

Cohorts

All patients and controls examined in this study were part of cohorts from either Calgary or Edmonton, recruited in 2010 and 2011, respectively. All participants completed the De Paul Questionnaire [10] to gather demographic data and to elicit the Canadian Consensus Criteria (CCC) for ME/CFS as established by Carruthers *et al.* [1]. Moreover, all participants were screened according to the Fukuda criteria [2]. Two participants did not meet the CCC and one participant did not meet Fukuda criteria, but all three were included on clinical grounds. The remainder of the CFS group met both the CCC and the Fukuda criteria. Healthy controls who showed more than one symptom of ME/CFS at moderate or greater severity were excluded. The CFS group (58 individuals) had a mean age of 48.9±10.1 years and 90% were female, compared to the healthy control group (57 individuals) with a mean age of 47.6±10.6 years and 89% female, reflecting the higher prevalence of the disease amongst women. A documented infectious onset could be reported by 59% of the CFS patients. Of the CFS patients, 93% have been sick for more than 2 years and 3% have been sick for 1–2 years, while 5% showed symptoms since childhood or adolescence.

Nested RT-PCR

For detection of XMRV/MLV sequences by nested PCR, RNA was extracted from 0.5 ml plasma using the QIAamp Ultrasens Virus Kit (Qiagen). The isolated RNA was immediately subjected to reverse transcription employing the Superscript III First-Strand Synthesis System for RT-PCR (Invitrogen). Culture supernatant from the XMRV-producing prostate cancer cell line 22Rv1 was used at a 10^{-5} dilution as a positive control for RNA isolation. For amplification of XMRV/MLV *gag* sequences, 5 µl of the transcribed cDNA were used for the first round of amplification with primers 419F (5′-ATCAGTTAACCTACCCGAGTCGGAC-3′) and 1154R (5′-GCCGCCTCTTCTTCATTGTTCTC-3′) [5] and HotStart-IT FideliTaq Master Mix (USB) with the recommended component volumes. The amplification was initiated by incubation for 4 min at 94°C, followed by 40 cycles of 1 min at 94°C, 1 min at 57°C and 1 min at 72°C, and a final incubation for 10 min at 72°C. Nested PCR was performed under the same conditions for 45 amplification cycles with 5 µl of the first round PCR product and two different primer pairs, Gag-I-F (5′-TCTCGAGATCATGGGACAGA-3′) and Gag-I-R (5′-AGAGGGTAAGGGCAGGGTAA-3′) or NP116 (5′-CATGGGACAGACCGTAACTACC-3′) and NP117 (5′-GCAGATCGGGACGGAGGTTG-3′), both of which have been shown to detect both XMRV and MLV sequences [6]. To determine the assay sensitivity, serial dilutions of a cloned fragment of XMRV *gag* [9] ranging from 1 to 100 copies/ µl were included in each PCR. The resulting PCR amplification products (730 bp for first round PCR and 413 bp or 380 bp for second round PCR, respectively) were analyzed by electrophoresis in 1.5% agarose gels. Any bands of approximately the correct size were excised and subjected to sequencing in order to determine homology to MLVs.

qRT-PCR

For qRT-PCR analysis, RNA was extracted from 100 µl of either whole blood or plasma using the Qiagen Viral RNA Mini Kit. The isolated RNA was subjected to reverse transcription by murine leukemia virus (MuLV) reverse transcriptase (Roche). The resulting cDNA was amplified in a real-time PCR reaction and quantified in a Roche LightCycler 480. Two different primer and probe sets were used for amplification of two distinct regions of the XMRV genome: primers XMRV-F2 5′-AACCTGATGGCAGATCAAGC-3′ and XMRV-R2 5′-CCCAGTTCCCGTAGTCTTTTGAG-3′ and probe FAM-AGTTCTAGAAACCTCTACACTC-BHQ1 for amplification of the XMRV integrase gene [11], and WPI primers Q445F 5′-GGACTTTTTGGAGTGGCTTTGTT-3′ and Q528R 5′- GCGTAAAACCGAAAGCAAAAAT-3′ and probe FAM-ACAGAGACACTTCCCGCCCCCG-BHQ1 for amplification of the XMRV-specific *gag* leader sequence [12] with FastStart Taq polymerase (Roche) in 45 amplification cycles of 95°C and 60°C for 30 sec each. Serial dilutions of a cloned fragment of XMRV *gag* [9] were used to produce standard curves (Fig. 1C). The sensitivity of the qRT-PCR assay was below 10^3 copies/ml plasma or whole blood.

Virus culture

DERSE (Detectors of Exogenous Retroviral Sequence Elements) indicator cells were developed at the National Cancer Institute by stable transfection of pBabe.iGFP-puro into LNCaP cells. pBabe.iGFP-puro is an MLV vector encoding puromycin resistance and a CMV promoter driven GFP reporter gene which is interrupted by an intron placed in sense direction relative of the vector and transcribed antisense to the vector mRNA. The intron interrupted GFP gene is only expressed after mobilization by an infecting gammaretrovirus for a second round of infection. After screening clonal cell populations, the most sensitive clones were chosen and designated as DERSE.Li-G cells. To test for the presence of infectious MLVs in patient plasma, DERSE.Li-G cells were inoculated with CFS patient plasma or control plasma. Cells were seeded 72 hours before infection with 3×10^4 cells/ml in 6-well plates. For spinoculation, the medium was removed and 300 µl fresh medium and 50 µl plasma were added per well. The plates were centrifuged at 1,200 rpm for 1 hour and 0.5 ml fresh medium was added. The inoculum was removed the next day and the cells were cultured in 2 ml fresh medium and monitored for GFP expression every 3 to 4 days for a total period of 3 weeks. As a positive control, culture supernatant from the 22Rv1 cell line (containing roughly 10^9 copies/ml as determined from the average of seven individual qPCR assays, data not shown) was used as an inoculum at 10^{-4}, and 10^{-6} dilution, respectively.

Serology

Western blot (WB) analysis was performed to detect anti-XMRV/MLV antibodies in CFS patient sera and healthy controls. Purified XMRV antigen from XMRV-infected DU145 prostate cells (C7) was denatured with SDS-PAGE sample buffer at 95°C for 10 min and analyzed by immunoblotting as previously described [9]. Seroreactivity was defined by reactivity to viral Env and/or Gag proteins of the expected size as seen in the positive control antisera (Fig. 2B).

Results

Whereas XMRV *gag* sequences were readily detectable in diluted 22Rv1 cell supernatants, XMRV and MLV were not detected in any of the patient plasma samples (Fig. 1A and B). The detection limit of the nested PCR assay was below 1 copy/ µl isolated RNA or 5 copies/reaction as determined by the detection of known amounts of XMRV plasmid DNA (Fig. 1B). The sensitivity of the qRT-PCR assay was below 10^3 copies/ml plasma or whole blood. Regardless of whether whole blood or plasma was tested, all human samples were negative for detectable amounts of XMRV nucleic acid (data not shown).

DERSE.Li-G cells inoculated with 22Rv1 supernatants showed a concentration-dependent GFP expression on day 7 and spread of the virus on day 21. GFP expression was not observed in any of the DERSE.Li-G cells inoculated with patient plasma (typical example shown in Fig. 2A).

Seroreactivity was defined by Western blot reactivity to viral Env and/or Gag proteins of the expected size as seen in the positive control antisera (Fig. 2B). None of the 115 human plasma reacted with the purified XMRV antigen indicating an absence of antibodies to XMRV/MLV in the samples (typical example shown in Fig. 2B). Increased background noise as observed for one

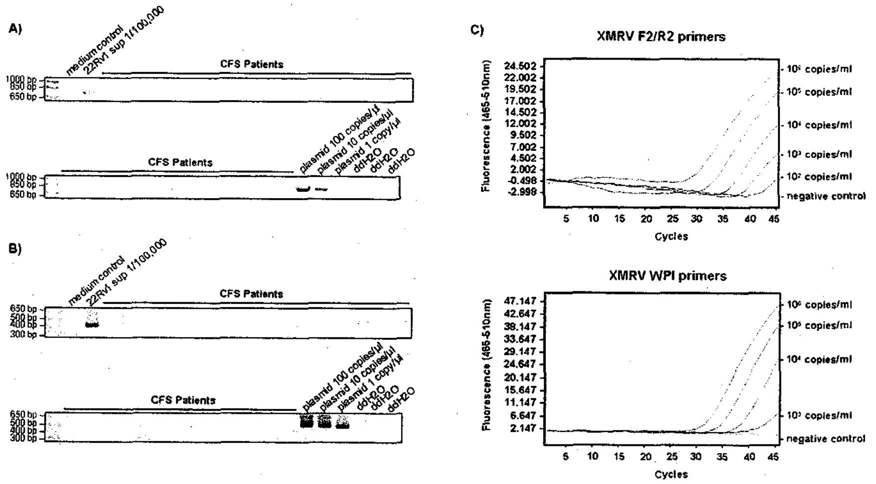

Figure 1. Failure of detection of XMRV nucleic acids in plasma and whole blood of CFS patients and healthy controls. *A)* First round PCR products of a representative number of RNA samples isolated from patient plasma using primers 419F and 1154R. A 10^{-5} dilution of 22Rv1 cell culture supernatant and three known concentrations of XMRV plasmid DNA were included as controls. *B)* Second round amplification products of nested PCR using primers Gag-I-F and Gag-I-R of samples shown in A). Identical results were obtained with primers NP116 and NP117 (see text, data not shown). The detection limit was below 1 copy/ µl isolated RNA or 5 copies/reaction. *C)* Results of qRT-PCR for XMRV plasmid control in serial dilutions ranging from 10^6 to 10^2 copies/ml as well as negative controls for both primer pairs used, F2/R2 (upper panel) and WPI (lower panel). All patient plasma and whole blood samples were found to be negative after a total of 45 amplification cycles (data not shown).
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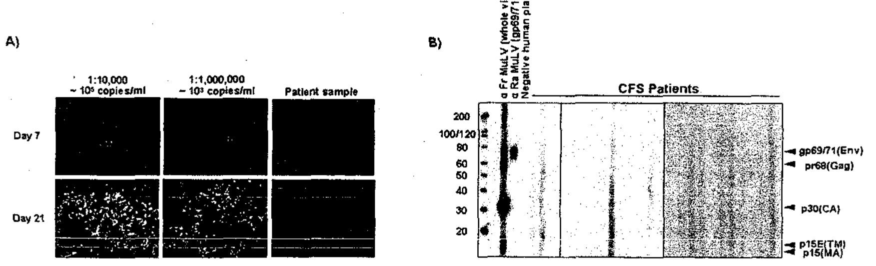

Figure 2. No evidence for infectious virus or XMRV-specific antibodies in plasma of CFS patients and healthy controls. *A)* GFP expression of DERSE.Li-G cells 7 days (upper panels) or 21 days (lower panels) after spinoculation with two different dilutions of 22Rv1 cell culture supernatants (10^{-4} and 10^{-6} dilution) or patient plasma. No GFP expression could be observed in any of the cells inoculated with human plasma. *B)* Immunoblotting of C7-purified XMRV antigen with patient plasma for detection of anti-XMRV/MLV antibodies. Representative WB results for CFS patients and healthy controls. Lane 1, anti-Friend MuLV whole virus, goat polyclonal antisera; lane 2, anti-Rauscher MuLV envelope, goat polyclonal antisera; lane 3, XMRV negative blood donor plasma. Locations of reactivity to specific viral proteins are indicated; Env (gp69/71), envelope; TM (p15E), transmembrane; MA (p15), matrix; Gag (pr68); CA (p30), capsid.
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of the CFS patient samples (lane 5, Fig. 2B) is most likely due to the presence of cross-reactive epitopes.

Discussion

In summary, we were unable to detect any evidence of XMRV or MLV infection in any of the 115 examined study participants, regardless of whether they were suffering from CFS or represented healthy controls. The 58 CFS patients enrolled in this study were carefully selected according to the Canadian Consensus Criteria for ME/CFS. Positively screened participants were only included if they showed symptoms in at least two categories of autonomous, neuroendocrine, and immune manifestations. The sensitivity of our assays reached copy numbers lower than 120 copies/ml of plasma for the detection of viral nucleic acids, and 10^3 copies/ml of plasma for the presence of infectious particles. While it is possible that XMRV and MLV are not predominantly blood-borne viruses and as such exist below the detection limit of most assays in plasma and whole blood, we believe that the assays used in this study are equally sensitive to those reported in previous positive studies. Moreover, our broad study design and the use of degenerate primers with specificity for highly conserved sequences in different MLV-like viruses and XMRV would have allowed us to identify nucleic acids, infectious particles, and antibodies for a number of related murine retroviruses. However, we could not detect any other murine retroviruses in any of our specimens, unlike the finding of MLV-like sequences reported by Lo *et al.* [6].

CFS patient cohorts have been tested for the presence of XMRV in the United States, Netherlands, Germany, China, and United Kingdom among others [4]. Being more aware of the possible risk of contaminants in commonly used laboratory reagents [13], none of these studies were able to reproduce the initial findings. Moreover, repeated testing of CFS patients previously reported to be infected with XMRV in the initial study performed by Lombardi *et al.* failed to detect any signs of XMRV infection in these patients [8]. On the contrary, it is now becoming increasingly clear that XMRV found in the prostate cancer cell line 22Rv1 originated from recombination of two MLVs present in the mouse strains used for passaging of the initial prostate cancer xenograft [14]. The fact that the viral sequences initially identified in prostate and CFS samples are virtually identical to those found in 22Rv1 cells [15] suggests that the assumed association of XMRV with human diseases is due to sporadic laboratory contamination. Moreover, differential handling of patient samples compared to controls can introduce bias and was therefore carefully avoided in this study. Two independent studies could show that handling of human samples in laboratory environments with abundant endogenous MLV proviruses can lead to the false detection of XMRV/MLV-like sequences due to contamination as proven by PCR detection of the highly abundant intracisternal A-type particle (IAP) long terminal repeat in the same samples [16,17]. In the light of the accumulating evidence for the artefactual origin of XMRV and the high burden of MLV-like DNA contamination the initially reported connection of XMRV and prostate cancer is now being ruled out as well [18]. Thus, although XMRV was found to infect and replicate in a variety of human cells, natural XMRV/MLV infection of humans has not yet been reproduced and is believed to be a false-positive result from mouse DNA and/or MLV-contaminated PCR reagents [13]. This study examines a possible association of XMRV and chronic fatigue in a Canadian patient cohort and is consistent with a number of recently published reports declaring no evidence for the presence of MLV-like viruses in any human subjects. In conclusion, while this study and others fail to support an association between XMRV and CFS, they highlight the urgent need for further research into the root causes of CFS.

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Use of trade names is for identification only and does not imply endorsement by the U.S. Department of Health and Human Services, the Public Health Service, or the Centers for Disease Control and Prevention. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Author Contributions

Conceived and designed the experiments: DLT ES TL YZ WMS MH GS. Performed the experiments: IS LM KL ST HJ DH DMS ML AL JL. Analyzed the data: IS TL WMS MH GS. Wrote the paper: IS MH GS.

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文献6

The scientific method at work: xenotropic murine leukemia virus–related virus is neither a cause of chronic fatigue syndrome nor a threat to the blood supply

Matthew S. Karafin and Susan L. Stramer

Sir Francis Bacon (1561-1626), the Lord Chancellor of England, was the first to provide a documented philosophical method for investigating a natural phenomenon. This method later became known as "the scientific method." Unlike many before him, he suggested that our understanding of the world should be based on data, rather than faith or dogma. Moreover, his method required that our understanding of the world be provisional, with the hope that our current understanding of natural phenomena would eventually be replaced by better science. The events that have transpired over the past 2 years regarding the clinical relevance of xenotropic murine leukemia virus–related virus (XMRV) have shown that the method of scientific investigation first described 400 years ago is very much alive and well today.

The scientific method, as envisioned by Sir Francis Bacon, starts with observation. Urisman and colleagues[1] first observed that XMRV is associated with human disease in 2006, finding that 40% of men with prostate cancer and a low activity variant of RNase L, an enzyme involved in the interferon-induced antiviral response, were infected with this virus. Subsequently in 2009 and 2010, two groups of investigators also described finding XMRV or related sequences of polytropic murine leukemia viruses (MLVs) in association with chronic fatigue syndrome (CFS), a disease characterized by severe fatigue and other related symptoms lasting more than 6 months.[2,3]

XMRV is currently understood to be a retrovirus, but is unrelated to other well-described retroviruses such as human immunodeficiency virus (HIV) and human T-cell lymphotropic virus (HTLV). XMRV specifically is a member of the family Retroviridae, subfamily Orthoretrovirinae, and genus *Gammaretrovirus* (see AABB XMRV Fact Sheet http://www.aabb.org/resources/bct/eid/Pages/default.aspx). XMRV is the first gammaretrovirus to be found in humans, and data indicate that it originated in mice after the recombination of two murine proviruses.[4] Virions are 80 to 100 nm in diameter, consisting of an envelope, nucleocapsid, and a nucleoid with a linear dimer of positive-sense, single-stranded RNA.[5]

From these initial observations, it was hypothesized that this virus could be causally related to both prostate cancer and CFS. Moreover, the finding of viral sequences in the blood of healthy controls in two studies[2,3] led to the concern that this virus could be transfusion transmitted, and thus the national blood supply could be at risk. Specifically, on June 18, 2010, the AABB issued a bulletin to its membership from its Interorganizational Task Force on XMRV that patients diagnosed with CFS be discouraged from donating blood. Consequently, the American Red Cross and a number of other blood donor centers started to offer educational information about CFS and have requested voluntary deferral of donors who ever have had a medical diagnosis of this debilitating condition.[6]

While the risk of transmission of XMRV by blood products was unknown at the time of release of this bulletin, the recommendation of the AABB Interorganizational Task Force was reasonable based on the initial hypothesis that CFS could have an infectious origin. First, XMRV is a gammaretrovirus, a genus that contains known animal pathogens (see AABB XMRV Fact Sheet). As other retroviruses, such as HIV and HTLV, are transfusion transmitted, it was plausible that an emerging retrovirus, such as XMRV, could also be transmitted by blood. Second, studies indicated that XMRV was physically present in blood. A rhesus macaque model of XMRV previously demonstrated that the virus can infect lymphoid cells, several tissues, and organs even though circulation of free virus was minimal.[7] Moreover, Lombardi and colleagues[2] found XMRV infection in the lymphocytes of the CFS patients studied. As lymphocytes are present in transfused cellular blood components, such as platelet products, red blood cells, and granulocytes, there is a substantial risk that this virus could be transmitted by transfusion.

The hypothesis that XMRV was a cause of human disease did not, however, withstand confirmation by other researchers who were investigating the same relationship. One review found that XMRV has been detected in 0% to 27.5% of prostate cancer patients in 12 studies.[5] Moreover, while the two original positive CFS studies found that XMRV/MLV sequences were present in up to 86.5% of CFS patients and up to 7% of healthy controls including blood donors,[2,3] as of 2011, at least 30 subsequent studies failed to reproduce these findings in CFS or other patient groups (see AABB XMRV Fact Sheet table). Additionally, these studies have failed to demonstrate a relationship between XMRV/MLV and CFS even with highly sensitive methods modeled after those described by Lombardi and coworkers,[2] including various molecular, serologic, and culture procedures, as well as enrolling many of the same CFS patients for study whose samples were previously identified as XMRV or MLV positive.[8-11]

Originally, several hypotheses were posed to explain the wide variations between study findings, including differences in cohort selection, testing methods, or sample source, preparation, and storage.[6,10,12] However, recent data indicate that laboratory, sample, and/or reagent contamination are the most likely causes for the results in those studies with positive findings.[4,8,9] The Scientific Research Working Group (SRWG) of the NHLBI, including several key authors of the original article associating XMRV/MLVs with CFS, sent samples in a blinded fashion to nine different laboratories, all with research tests for XMRV including detection of nucleic acids (11 laboratories), antibody (five laboratories), and virus following culture (three laboratories). They found that current testing methods do not reproducibly detect XMRV, even in blood samples from patients that were reported to have XMRV infection previously (including 14 with CFS) or in XMRV-negative, healthy controls.[11] Of note in this study, only the two laboratories associated with Lombardi and colleagues[2] obtained positive results for any samples (excluding the spiked positive controls); however, these laboratories found positive DNA and antibody results in healthy controls at the same rate as positive results in CFS patients with the additional caveat that positive patient results were inconsistent. Perhaps most importantly, Paprotka and colleagues[4] proposed that XMRV originated as the result of a laboratory recombination event involving two mouse proviruses that occurred during the serial passage of a human prostate cancer xenograft (CWR22) in nude mice in the 1990s. When aligned, these two proviruses were identical to the sequence of XMRV. Thus, the authors concluded that XMRV is not a real human pathogen and that positive findings were the result of contamination by a laboratory-derived virus.[4] In a subsequent related study, it was shown that both proviruses occurred in laboratory mice but not in wild strains of mice, and no laboratory mouse strain could harbor XMRV replication due to the lack of the required receptor in laboratory mice, indicating that the xenografted human tumor cells were required for XMRV propagation.[13] In addition, the genetic distance among *env* and *pol* sequences from the persistently XMRV-infected prostate cell line, 22Rv1, derived from the CWR22 xenograft, exceeds that of patient-associated sequences, suggesting laboratory contamination versus human infectious transmission. Thus, XMRV derived from the 22Rv1 cell line is the genetic ancestor of all subsequent isolates from CFS or other patients.[14]

XMRV also does not appear to be a concern for blood recipient safety. Studies have recently demonstrated that XMRV would not be able to persist or replicate in human blood due to cell-mediated antiviral pathways.[9] A large recent study further demonstrated that no XMRV antibody could be detected from 17,249 blood donors or recipients, including 13,399 US blood donors from six different regions and 3741 donors linked to 109 recipients of which 830 samples were tested over a 2-year period. A positive antibody result required reactivity to three different XMRV proteins, and the tests used were the same as those used by the SRWG and represented those tests that were automated and could be used for blood donation screening if needed. Since RNA could also not be found in any recipient or any donor with isolated antibody reactivity, the study concludes that XMRV is not a current threat to blood safety.[15]

The mounting negative findings failing to associate XMRV/MLV with human disease, and now documentation of XMRV as a laboratory artifact, prompted the Editor of *Science* to call for a retraction of the 2009 publication by Lombardi and colleagues in an expression of concern.[16] However, the authors of the original study have not agreed to retract their original work entirely; a partial retraction initiated by one author, and signed by the other authors, has resulted in removal of the polymerase chain reaction data due to sample contamination with XMRV plasmid DNA.[17] Taken together, the scientific data to date indicate that XMRV/MLV is neither a human pathogen nor a risk to the national blood supply (see reviews detailing the chronology of events and investigations of potential XMRV disease associations since October 2009[18-20]). Most recently (December 23, 2011), the Editor-in-Chief of *Science* has issued an editorial retraction of Lombardi et al.[2] due to the inability of multiple laboratories to reproduce the study findings, including those of the original authors; questions of quality control related to a number of specific reported experiments; and an overall loss in confidence in the validity of the conclusions.[21] This was followed by a retraction of the Lo et al. manuscript[3] by the authors on December 27, 2011. One study examining the

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TRANSFUSION 2012;52:222-225.

potential link of XMRV with CFS is still pending. The study is sponsored by the NIAID and led by Ian Lipkin of Columbia University; this likely will be the last major study investigating the disease potential of these agents.

Thus, over the past 2 years, XMRV has transformed from an agent of potential human disease association, and a possible threat to the national blood supply, to a laboratory contaminant without a current threat to humans. This revolution of ideas regarding XMRV could only have been made possible by the scientific method. Sir Francis Bacon aptly described the scientific process with the following metaphor:

> Those who have handled sciences have been either men of experiment or men of dogmas. The men of experiment are like the ant; they only collect and use: the reasoners resemble spiders, who make cobwebs out of their own substance. But the bee takes a middle course; it gathers its material from the flowers of the garden and the field, but transforms and digests it by a power of its own. Not unlike this is the true business of philosophy; for it neither relies solely or chiefly on the powers of the mind, nor does it take the matter which it gathers from natural history and mechanical *experiments and lay it up in the memory whole,* as it finds it; but it lays it up in the understanding altered and digested. Therefore from a closer and purer league between these two faculties, the experimental and the rational (such as has never yet been made) much may be hoped. (Book 1, Aphorism 95)[22]

Just as Sir Francis Bacon predicted, the astute combination of a rational evaluation of current knowledge with rigorous experimental observation allows the scientific community to move seamlessly from an unproven working hypothesis to a result based on the synthesized accumulation of data disproving the hypothesis. In conclusion, the scientific process remains to this day a powerful tool to understand our natural world, and XMRV clearly demonstrates the potency of a 400-year-old method. Sir Francis Bacon would be proud.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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資料5

ひと、くらし、みらいのために
厚生労働省
Ministry of Health, Labour and Welfare

Press Release

平成24年2月24日
医薬食品局血液対策課
（担当・内線）課長補佐　伯野（2905）
血液安全係長　松本（2908）
（代表電話）03(5253)1111
（直通電話）03(3595)2395

報道関係者　各位

フィブリノゲン製剤納入先医療機関の追加調査について

平成16年12月9日に公表したフィブリノゲン製剤納入先医療機関を対象として、平成 19 年 11 月7日付で実施した追加調査の結果について、**平成 24 年2月 10 日**までに回収した医療機関からの回答を取りまとめた状況をお知らせいたします。

（参考）　C型肝炎ウイルス検査受診の呼びかけ（下記の厚生労働省ホームページにリンク）
http://www.mhlw.go.jp/houdou/2008/01/h0117-2/index.html

1　回答状況

（1）追加調査実施期間　　平成 19 年 11 月7日～12 月5日（※1）
（ただし、現在も回収中）

（※1）（1）の調査以降も毎年度、元患者の方へのお知らせ状況等について再度調査を行っており、（3）回答施設数以降はそれらの結果を反映したものである。

（2）追加調査対象施設数　　医療機関　6,610 施設
（平成16年公表施設のうち、所在地等が不明であった施設を除いた医療機関）

（3）回答施設数
- 平成 16 年公表時に存続していた 5,397 施設のうち、5,291 施設（98%）から回答があった。
- なお、このほか平成 16 年公表時に廃院等していた 1,213 施設のうち、508 施設から回答があった。

2　主な調査結果

（1）投与の年月について回答があった医療機関数と元患者数

医療機関数	944 施設
元患者数	14,454 人　（投与年別は別表）

（2）上記以外に、過去に投与の事実をお知らせしたという記録が残されているが、現在では投与の年月は特定できないとする回答があった医療機関数と元患者数

医療機関数	104 施設
元患者数	332 人

（3）（1）と（2）の合計

医療機関数	1,024 施設（※2）
元患者数	14,786 人

（※2）厚生労働省ホームページ「C型肝炎ウイルス検査受診の呼びかけ（フィブリノゲン製剤納入先医療機関名の再公表について）」の公表医療機関等リスト上の該当医療機関の「備考」欄に、「フィブリノゲン製剤を投与されたことが判明した元患者の方がいるとの報告あり。」と記載した。

（4）元患者の方への投与の事実のお知らせの状況

		元患者数
お知らせした		8,762人　（59%）（※3）
お知らせしていない		6,024人　（41%）
理由	投与後に原疾患等により死亡	1,967人　（13%）
	連絡先が不明又は連絡がつかない	2,855人　（19%）
	肝炎ウイルス検査の結果が陰性	468人　（ 3%）
	今後お知らせする予定である	292人　（ 2%）
	その他（未記入含む）	442人　（ 3%）
合　計		14,786人

（※3）元患者の方に一人でも投与の事実をお知らせした医療機関は 846 施設であった。

(5)診療録等の保管状況

平成6年以前の診療録等が次のいずれかにより保管されている施設数
（括弧内は調査対象施設数に対する割合）

2,045 施設（31%）（※4）

（内訳）（※5）

診療録（カルテ）	1,498 施設	(23%)
手術記録あるいは分娩記録	1,578 施設	(24%)
製剤使用簿	136 施設	(2%)
処方箋	144 施設	(2%)
輸液箋あるいは注射指示箋	276 施設	(4%)
レセプトの写し	83 施設	(1%)
入院サマリーあるいは退院サマリー	296 施設	(4%)
その他の書類	296 施設	(4%)

（※4）平成 16 年の調査では「昭和 63 年6月 30 日以前にフィブリノゲン製剤を投与した記録（診療録、使用簿など）が保管されていますか。」との設問であったのに対し、今回の調査では、「平成6年以前のカルテ等の各種書類が保管されていますか。」との設問であったため、保管していると回答した施設の割合が異なったものと思われる。

（※5）厚生労働省ホームページ「C型肝炎ウイルス検査受診の呼びかけ（フィブリノゲン製剤納入先医療機関名の再公表について）」の公表医療機関等リスト上の「カルテ等の有無」欄に、平成6年以前のカルテ等の記録が一部でも保管されている場合、△印を付していたが、さらに保管されている記録の保管期間、保管状況等を記載した。

（別表）

投与の年月について回答があった元患者数の投与年別の内訳

投与年	人数
昭和　39年	0人
40年	7人
41年	9人
42年	12人
43年	16人
44年	19人
45年	20人
46年	23人
47年	30人
48年	48人
49年	61人
50年	58人
51年	72人
52年	93人
53年	131人
54年	202人
55年	336人
56年	443人
57年	575人
58年	982人
59年	1,530人
60年	1,816人
61年	2,495人
62年	3,037人
63年	1,736人
平成　元年	244人
2年	181人
3年	108人
4年	63人
5年	53人
6年	44人
計	14, 454人

追加資料1

平成24年3月14日
薬事・食品衛生審議会
血液事業部会運営委員会
提出用資料

日本赤十字社

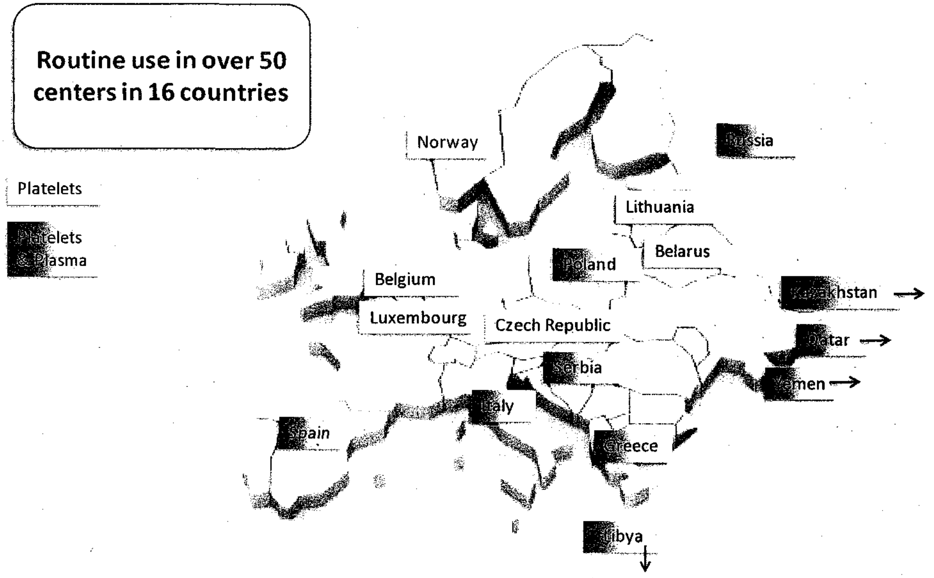
血小板製剤に対する感染性因子低減化(不活化)技術の導入準備について(追加報告)

1. 欧州における導入状況について

①リボフラビン法(Mirasol)

Growing Adoption of Mirasol in Europe & Middle-East

Routine use in over 50 centers in 16 countries



(TerumoBCT 社提供資料)

現在、欧州を中心に 16 カ国でリボフラビン法処理血小板がルーチンで使用されており、そのうち 10 カ国では血漿も処理されている。なお、ベルギー(アモトサレン法が導入されていない北部地方)では、低減化処理キットの仕様変更のため、現在医療機関への供給は一時中断しているが、本年夏以降に再開される予定である。

このうちの数カ国での低減化処理血小板製剤の市販後調査が実施中であるが(次ページ)、現時点において低減化処理に起因する重篤な副作用等の報告は受けていない。

Table 6. Clinical evaluations [50, 88–91]

Site	Number of transfusions	24-hour CCI	Adverse events	Neoantigen formation
MIRACLE trial – reference group	160	9,886	3 (severe*)	ND
MIRACLE trial – Mirasol group	175	6,676	2 (severe*)	none
Serbia	87	5,166	none	none
Poland	4,328	ND	12 (grade 1#)	none
Spain	44	6,351	none	ND
Mirasol evaluation program	368	ND	none	ND

*The following categories of adverse event severity were used: *Mild:* Awareness of a sign or symptom that does not interfere with the patients usual activity or is transient, resolves without treatment and with no sequelae. *Moderate:* Interferes with the patient's usual activity and/or requires symptomatic treatment. *Severe:* Symptom(s) causing severe discomfort and significant impact of the patients usual activity and requires treatment.

#Severity of reactions followed the classification suggested by the International Hemovigilance Network. *Grade 1:* The recipient required no more than discontinuation of transfusion and symptomatic management. No long-term morbidity. *Grade 2:* The recipient requires in-patient hospitalization or prolongation of hospitalization due to hypotension, or hypotension led directly to long-term morbidity.

Transfus Med Hemother 2011;38:8–18

②アモトサレン法(Intercept)

INTERCEPT Routine Centers per Country

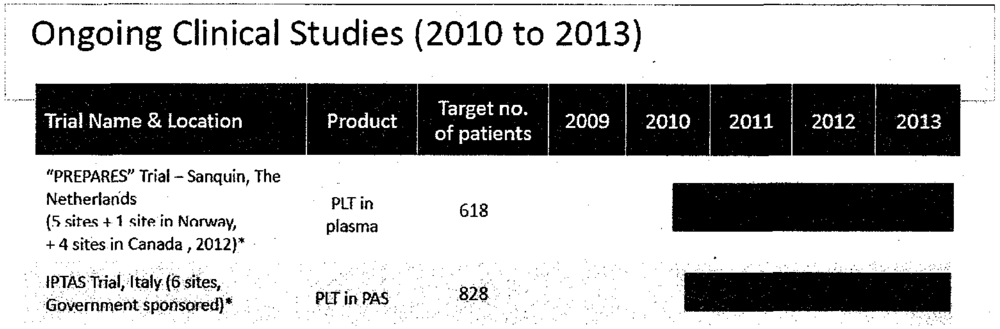
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1	Austria	1	1	0	0
2	Belgium	5	5	4	4
3	France DOM/TOM	4	4	1	1
	France	10	1	10	1
4	Germany	1	0	1	0
5	Greece	1	1	0	0
6	Italy	14	14	7	7
7	Kazakhstan	11	9	9	7
8	Kuwait	1	1	0	0
9	Norway	2	2	0	0
10	Portugal	2	2	0	0
11	Russia	18	12	12	6
12	Slovenia	1	1	0	0
13	Spain	12	12	2	2
14	Sweden	7	7	0	0
15	Switzerland	13	13	0	0
	Total	**103**	**85**	**46**	**28**

(Cerus 社提供資料)

15カ国でアモトサレン法がルーチンで使用されており、そのうち14カ国では血小板も処理されている。これらの国のうち、スイスは2011年11月に全ての血液センターでアモトサレン法で処理した血小板製剤の製造を開始した。また、フランス本土では血小板は1センターのみで変化はないが、メチレンブルー処理血漿についてヘモビジランスの結果、アレルギー反応が多いとの理由でフランス保健製品衛生安全庁(AFSSAPS)が本年度中の使用中止を勧告したため、血漿にアモトサレン法を導入するセンターが増加した。一方、ドイツではアモトサレン法処理血小板製剤が早い段階で承認されているが、現時点で血小板製剤にルーチンで使用しているセンターは無い。

なお、上記表には記載されていないが、本年2月1日にイスラエルがアモトサレン法処理血小板と血漿を承認したとのプレスリリースが発出されている。

2. 実施中の主な臨床試験について



Ongoing Clinical Studies (2010 to 2013)

Trial Name & Location	Product	Target no. of patients	2009	2010	2011	2012	2013
"PREPARES" Trial – Sanquin, The Netherlands (5 sites + 1 site in Norway, + 4 sites in Canada , 2012)*	PLT in plasma	618		■	■	■	■
IPTAS Trial, Italy (6 sites, Government sponsored)*	PLT in PAS	828		■	■	■	■

(TerumoBCT 社提供資料)

①PREPARES

オランダのサンキン財団を主体としてオランダ、ノルウェー、カナダで実施されている、全血採血由来のリボフラビン処理血小板製剤と通常の血小板製剤のランダム試験(患者登録終了予定:2013年末)

②IPTAS

イタリア政府が実施している、添加液(PAS)で置換してリボフラビン法またはアモトサレン法で処理した血小板製剤と、通常の血小板製剤との比較試験(同:2014年末)

PREPARESはメーカー主導ではなく症例数が多いこと、また、IPTASは同じプロトコールで、リボフラビン法及びアモトサレン法で処理した血小板の臨床試験が実施されることから、これらの試験の動向を注目しているところである。

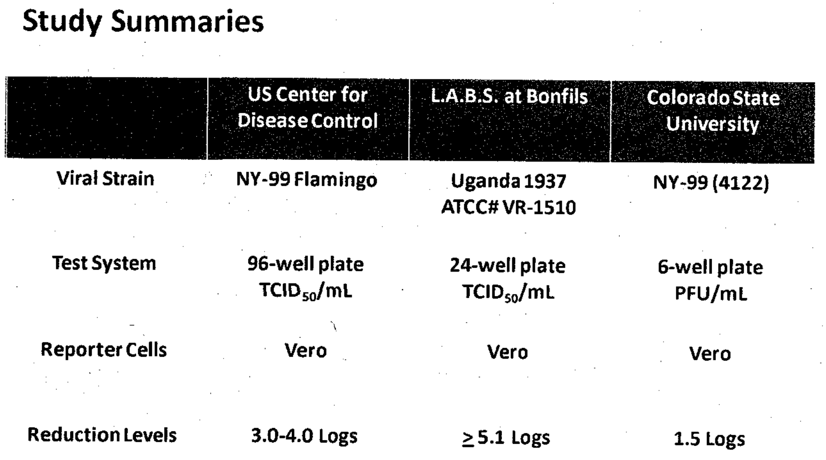
3. 日本赤十字社における検討状況等について

①リボフラビン法(Mirasol)

・CaridianBCT社がテルモ社に買収されTerumoBCT社となったことにより、テルモ社の有する安全性部門や研究開発部門の協力を得ることができるようになった。現在、今後の承認申請に必要となるAFSSAPSに提出された安全性に関するデータの整理を依頼している。

・日本赤十字社において、製剤としての規格や品質について決定するための試験を実施しているが、平成22年3月の本委員会に報告した凝集塊の発生という問題については、未だ解決できていない。日本赤十字社の検討ではかなりの確率で凝集塊が発生するのに対し、他の国では類似した凝集塊の発生はないとの報告を受けている。現在、TerumoBCT社と密接に連絡を取りながら、解決策を探っているところである。

・新興再興感染症対策としてウエストナイルウイルス(WNV)の低減化について検討したところ、日本赤十字社のデータとTerumoBCT社のデータに乖離があることが明らかとなった。乖離の原因を特定するため、種々のWNV株について低減化率を測定した結果、細菌と同様、使用した株により低減化率に差が生じることが明らかとなり、ヒトから分離されたUganda1937株に対しては、十分な低減化効果があることが再確認された。



Study Summaries

	US Center for Disease Control	L.A.B.S. at Bonfils	Colorado State University
Viral Strain	NY-99 Flamingo	Uganda 1937 ATCC# VR-1510	NY-99 (4122)
Test System	96-well plate $TCID_{50}$/mL	24-well plate $TCID_{50}$/mL	6-well plate PFU/mL
Reporter Cells	Vero	Vero	Vero
Reduction Levels	3.0-4.0 Logs	≥5.1 Logs	1.5 Logs

(TerumoBCT 社提供資料)

②アモトサレン法（Intercept）

- BioOne 社が精算されたため、現在、Cerus 社と直接情報交換を行っている。
- アモトサレン法の処理キットには、日本における需要の 8 割以上を占める 10 単位製剤の規格に合うものがなく、また、アモトサレンの吸着除去処理に長時間を要することなどから、現状の処理キットを日本に導入することは困難であるが、10 単位製剤に最適化した処理キットの開発について検討したいとの話があった。

4. 今後の予定