Porcine red blood cells express a polyagglutinable red blood cell phenotype

The shortage of human blood and organs has led to increased interest in animal substitutes. In both transplantation and transfusion, the greatest roadblock to the use of animal tissues is the expression of xenoantigens. A major xenoantigen in pigs is linear B (Galα1-3Galβ1-4GlcNAc-R), a cross-reactive, B-like antigen expressed on red blood cells (RBCs) and other tissues that is recognized by naturally occurring antibodies present in human serum.[1,2]

Recently, Rouhani and colleagues[2] reported their experience with porcine RBCs (pRBCs) from genetically engineered pigs that lack linear B (α1,3-galactosyltransferase knockouts [αGal–]).[2] Relative to wild-type controls, pRBCs from αGal– animals showed a marked decrease in hemolysis and immunoglobulin G and immunoglobulin M binding after incubation in human serum. Although decreased, antibody binding to αGal– pRBCs still occurred, suggesting the presence of other xenoantigens. This was confirmed by in vivo studies in baboons, which showed rapid clearance (<5 min), with evidence of hemolysis, for αGal– and wild-type pRBCs. It was hypothesized that pRBCs were removed by hepatic macrophages, possibly via an endogenous lectin on Kupffer cells.

We would like to share our serologic results using lectins of defined specificity, which showed that pRBCs express a polyagglutinable phenotype that is independent of linear B expression. Several years ago, one of us (J.L.S.) routinely typed pigs for the transplant surgery research program. Washed pRBCs were forward typed as group A or "O" with commercial murine monoclonal antibodies (MoAbs) by standard-tube agglutination (Table 1). No animal typed as group B. The presence of linear B was confirmed in a small cohort of animals with *Griffonia simplicifolia* (GS1), which recognizes terminal α-galactose.[3]

As shown in Table 1, regardless of ABO type, 99 percent of all animals tested were T-antigen–positive (*Arachis hypogaea*, PNA). Only three animals were PNA-negative, including two littermates, indicating that PNA+ was an autosomal dominant phenotype. In addition to PNA, 90 to 100 percent of animals tested were also positive with *Glycine soya* (SBA), *Salvia sclarea* (SSA), and *Salvia horminum* (SHA). Most animals were also positive with the anti-A lectins *Dolichos biflorus* (DBA) and *Phaseolus lunatus* (LBA). Titration and adsorption studies suggested

TABLE 1. Hemagglutination of pRBCs by blood group active MoAbs and lectins

	Reagent specificity		Serologic typing of pRBC*			
					Results by pig ABO type	
Reagent	Carbohydrate epitope	Blood group	Total number of pigs tested	Number (%) of pigs positive	Group O†	Group A†
Anti-A MoAb†	GalNAcα1-3(Fucα1-2)Gal	A	401	130	271†	130
Anti-A human‡	GalNAcα1-3(Fucα1-2)Gal	A	20	20	10	10
Anti-B MoAb†	Galα1-3(Fucα1-2)Gal	B	401	0	0	0
G. simplicifolia (GS-1)	αGal	B, linear B	29	29	NA§	NA
Ulex europeaus (UEA-1)	αFuc	H (O)	146	0	0	0
D. biflorus (DBA)‖	αGalNAc	A_1	411	391 (95)	166 (93)	68 (94)
Strong positive (3-4+)				375 (91)	156 (88)	67 (93)
Weak positive (1-2+)				16 (4)	10 (5)	1 (1)
Negative (±-0)				20 (5)	12 (7)	4 (6)
Phaseolus lunatus (LBA)¶	αGalNAc	A	29	24 (83)	NA	NA
A. hypogaea (PNA)‖	Galβ1-3GalNAc	T	264	261 (99)	175 (99)	71 (98)
Strong positive				256 (97)	173 (97)	68 (94)
Weak positive				5 (2)	3 (2)	3 (4)
Negative				3 (1)	2 (1)	1 (1)
G. soya (SBA)	GalNAc	T, Tn, CAD	29	29 (100)	NA	NA
S. sclarea (SSA)¶	GalNAc	Tn	29	29 (100)	NA	NA
S. horminum (SHA)¶	GalNAc	Tn, CAD	29	24 (83)	NA	NA
Bauhinia purpurea (BPA)¶	Gal/GalNAc	T, lactose	29	26 (90)	NA	NA

* Data are reported as number (%).
† Porcine RBCs were forward typed with anti-A and anti-B murine MoAbs according to manufacturer's instructions for human RBCs. RBCs that did not agglutinate with anti-A and anti-B MoAbs were interpreted to be group O (n = 271 animals). Note that no pRBCs were agglutinated by anti-H lectin, UEA-1.
‡ Human anti-A was extensively adsorbed using group O pRBCs to remove anti-αGal.
§ NA = not available.
‖ Reactions of pRBCs were classified as strong positive (3-4+), weak positive (1-2+), and negative (±-0) based on the strength of agglutination with the lectins DBA and PNA.
¶ Variable agglutination strength between animals (range, 1 ± 3+).

TABLE 2. Comparison of pRBCs and polyagglutinable human RBC phenotypes[3]

	Serologic reactivity with lectins* and AB human serum						
	Human serum	DBA	PNA	SBA	SSA	SHA	GS1
pRBCs	+	+	+	+	+	+	+
T^3	+	−	+	+	−	−	−
Tk^3	+	−	+	−	−	−	−
Tn^3	+	+	−	+	+	+	+
CAD^3	+	+	−	−	−	+	−

* See Table 1 for lectin names and specificity.

that DBA recognized a unique, cross-reactive A-like antigen on pRBCs. The latter may represent the A^p antigen defined in older literature. There was no correlation between PNA and DBA positivity ($p = 0.24$, chi-square).

These studies support the contention of Rouhani and coworkers[2] that pRBCs may express additional carbohydrate epitopes, capable of binding naturally occurring antibodies and/or endogenous lectins, to affect pRBC hemolysis and removal. A similar conclusion was reported by Gautreau and associates[4] who observed strong hemagglutination of pRBCs with human serum depleted of anti-A, anti-B, and linear B agglutinins.[4] Finally, multiple glycolipid antigens in pigs and sheep were recently identified as potential novel xenoantigens, as evidenced by their reactivity with AB human serum that had been depleted of Forssman and αGal antibodies by immune adsorption.[1]

Our results show that pRBCs share features with many polyagglutinable RBC phenotypes (Table 2). A known senescent antigen on human RBCs, T-antigen is clinically associated with hemolysis and shortened in vivo survival.[3] Shortened RBC survival and hemolysis are also observed in patients with the Tn syndrome.[3] Interestingly, the Tk antigen has been implicated in the short survival of platelets stored at 4°C. Cold storage is associated with the exposure and clustering of Tk antigen present on glycoprotein-Ibα, which is subsequently recognized by $\alpha_M\beta_2$ integrin receptor (complement receptor 3) on hepatic macrophages.[5]

In summary, these results emphasize the problems facing xenotransplantation. Genetically engineered animals, which lack linear B antigen and/or Forssman antigen expression, may still be quite antigenic owing to species-specific differences in the regulation and expression of other carbohydrate antigens. More research into the differentiation and regulation of cell surface glycosylation in humans and animals is needed.

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The above letter was sent to Rouhani et al.: Drs Dor, Rouhani, and Cooper offered the following reply.

We read with interest the letter by Swanson and Cooling,[1] written in response to our recent article,[2] in which they provide evidence that porcine red blood cells (pRBCs) express a polyagglutinable phenotype that is independent of α1,3-galactosyltransferase (αGal; linear B) expression. We fully agree with them that xenoantigenicity is still present in αGal gene-knockout pigs. As they provide evidence, other xenoantigens are present on pRBCs;[3] one such example has been demonstrated by Zhu and Hurst[4] to be *N*-glycolylneuraminic acid. Furthermore, knocking out the αGal gene may alter the glycosylation pattern on cell surfaces, as reported by Shinkel and coworkers,[5] resulting in exposure of neoantigens, which could lead to destruction of these pig cells by antibodies or other constituents of the host immune response. One potential partial solution to this problem would be to replace the αGal epitopes with another oligosaccharide (through transgenic expression of a human gene) that would not be bound by human antibodies, for example, that for the H(O) blood group oligosaccharide.[6,7]

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Travel-related hepatitis A detected by hepatitis A virus RNA donor screening

We read with interest the report by Gowland and coworkers[1] describing molecular and serologic tracing of transfusion-transmitted hepatitis A virus (HAV) between a blood donor and recipient. We describe the application of reverse transcription–polymerase chain reaction (RT-PCR) and phylogenetic analysis to tracing acute hepatitis A infections in two blood donors to a common source of HAV infection during their vacations in Egypt.

Within a 3-week period, two blood donations from different regions of Germany (Donor A, July 22, 2004; and Donor B, August 12, 2004) tested positive by HAV PCR during routine processing at the Red Cross Blood Transfusion Service West. Both donors were asymptomatic and neither had been vaccinated against hepatitis A. Both had recently returned from holiday trips to Hurghada, Egypt, where they stayed in different, but neighboring, Red Sea beach hotels. They were not deferred as blood donors, because Hurghada is not a known risk area.

Routinely, all blood donated at the Red Cross Blood Transfusion Service West is tested by PCR for HCV, human immunodeficiency virus-1 (mandatory by German Guidelines), hepatitis B virus, parvovirus B19 DNA, and HAV RNA in accordance with requirements of the plasma fractionation industry.[2] PCR screening of plasma is conducted using 96-sample minipools at the Laboratory of the Red Cross Blood Transfusion Service Baden-Wuerttemberg-Hessen by real-time PCR. PCR-positive minipools are traced back by chessboard-pooling and confirmed by single-donation PCR.

Additional testing for these two donors included quantitative PCR performed by one-tube RT-PCR (RealArt HAV LC RT-PCR kits, artus GmbH, Hamburg, Germany). Full-length RNA was reverse-transcribed and subsequently amplified and sequenced (DNA Cloning Service, Hamburg, Germany). Sequence data analysis was performed by CLUSTAL W algorithm. Quantification of HAV with the donors' fresh frozen plasma revealed titers of 2.5 EE2 IU per mL (Donor A) and 5.3 EE3 IU per mL (Donor B). The closest sequence match to GenBank was HAV strain 2F84 (97%) from a sporadic case in France in 1984.[3] Sequence analysis of both strains isolated from the plasma units revealed 100 percent homology, confirming a common source of their infections. Sequence alignment to known strains of HAV matched genotype 1B for both donations (Fig. 1). Typically, genotype 1A predominates in Germany.

The donors were informed about their test results, asked for follow-up blood samples, and interviewed by telephone to investigate the possible sources for their HAV infections. No follow-up sample was available from Donor A. His family doctor reported that he was hospitalized on August 5, 2004, because of jaundice, abdominal pain, fatigue, and loss of appetite. Hepatitis A was diagnosed and confirmed by the presence of anti-HAV immunoglobulin M (IgM) and immunoglobulin G (IgG). His apartase aminotransferase level was 11,120 U per L and his alanine aminotransferase level was 5,664 U per mL. He recovered in 2 weeks. A follow-up sample from Donor B from August 19, 2004, was positive for both anti-HAV IgM and IgG and HAV PCR. He was asymptomatic, but 9 days later, he developed fever, pain in the upper abdomen, arthralgies, and one episode of dark urine. This illness lasted 2 days. The specific source of their infections has

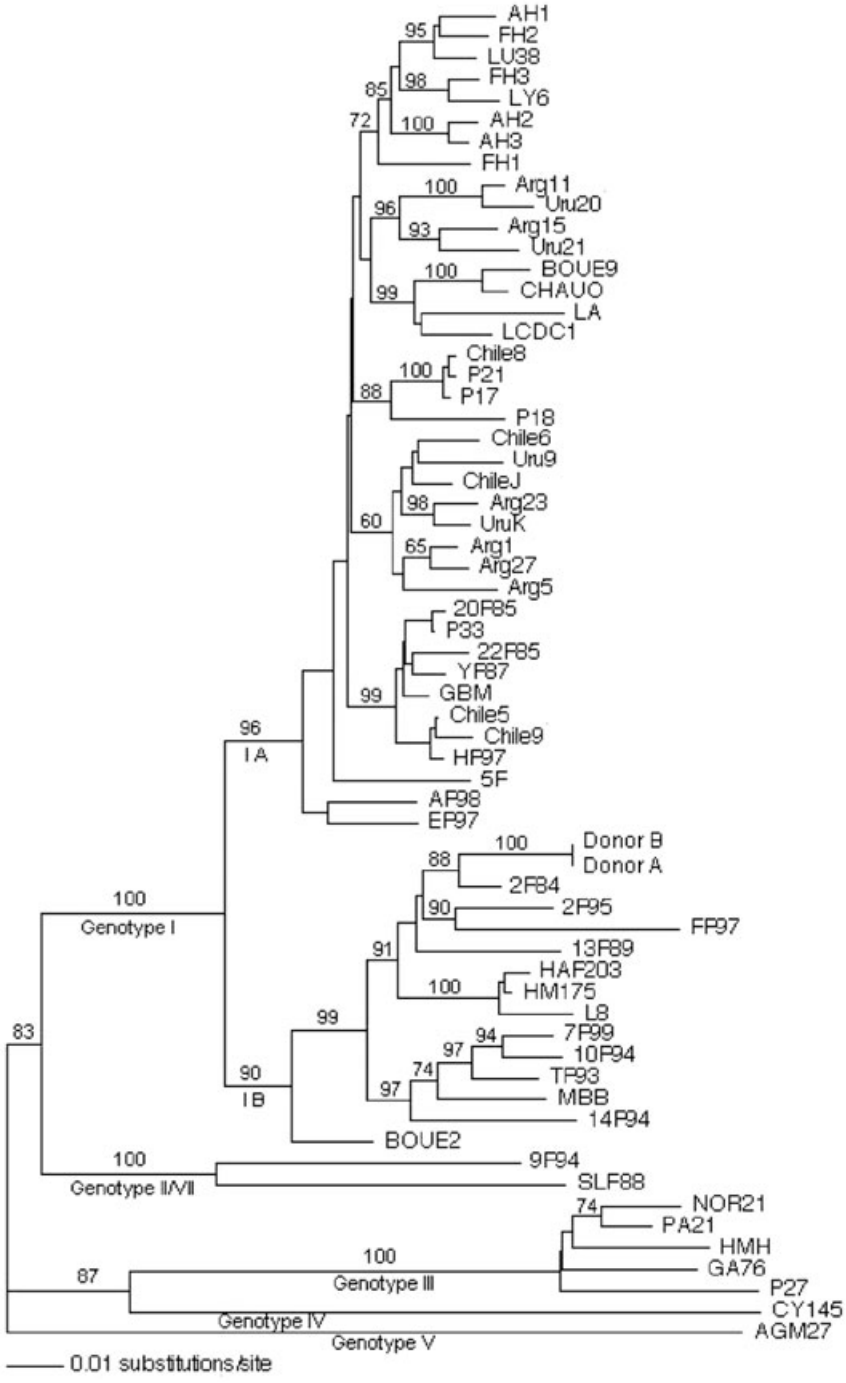

Fig. 1. Phylogenetic analysis of the two HAV strains isolated of the plasma unit (Donors A and B). Numbers at the branches indicate bootstrap percentage after 1000 replications of bootstrap sampling. The bar indicates genetic distance.

not been identified.[4] Four weeks after the donors departed, however, one of the hotels was identified as the source of more than 300 cases of hepatitis A among European tourists.[5]

This experience alerts us that there is a substantial risk for hepatitis A among nonvaccinated tourists not only in known regions of known high risk for hepatitis A, but also in certain areas where the risk has not been recognized. When blood is donated shortly after a stay in an HAV high-risk region, either testing donations by HAV NAT or a temporary deferral of donors (when common source outbreaks are recognized) is appropriate to prevent transfusion-transmitted infections.

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Lack of correlation between hepatitis B surface antigen and hepatitis B virus DNA levels in blood donors

Kuhns and coworkers[1] report a lack of correlation between hepatitis B surface antigen (HBsAg) and hepatitis B virus (HBV) DNA levels in chronic carriers of HBV. We would like to confirm this conclusion with data obtained in blood donors from Kumasi, Ghana, West Africa. In all cases, samples were stored at –20°C or below and transported in dry ice.

The first set of donors was tested in 1999 to 2000 with an HBsAg enzyme immunoassay (EIA; Abbott/Murex, Dartford, UK) that has a sensitivity of 0.2 ng per mL. Seroreactive deferred donor samples were confirmed with an alternative EIA (Biokit, Valencia, Spain) and tested for alanine aminotransferase (ALT) level (upper normal level, 40 IU/L). Forty donors from the same set were selected for having on two consecutive occasions, at least 1 month apart, an ALT level above 60 IU per L (range, 60-865; median, 185) as evidence of liver disease and entry criteria in a randomized clinical trial for treatment with a local, plant-derived drug.

There were 32 men and 8 women, ranging in age between 17 and 54 years (median, 24 years). Viral load was quantified using an in-house real-time PCR (Q-PCR) as previously described.[2] It ranged between 15 and 4.1×10^8 IU and mL. Twenty-two samples had a viral load of greater than 1×10^4 and were HBeAg-positive, whereas the other 18 samples were below that level and were anti-HBe–positive.

HBsAg was semiquantified in the following way. Plasma samples were serially diluted by a factor of 10 in saline, and each of the seven dilutions was tested with the HBsAg screening EIA. Reactivity was expressed as sample-to-cutoff ratio (S/CO). S/COs were then plotted against dilution on a bilogarithmic scale, and the two to four samples with a S/CO below maximum were used to derive a best-fit curve that intersected the base line of S/CO of 1. This intersection was considered as extinction dilution and taken as reflecting the concentration of HBsAg.

As shown in Fig. 1A, no correlation was found between viral load that reflects the viral replication and HBsAg endpoint dilution that reflects the level of synthesis of surface protein. All but one sample gave a positive signal with the EIA when diluted 1:100,000 or higher. Seven of eight samples containing less than 100 IU per mL HBV DNA (approximately 500 copies) gave a positive signal when diluted 1:1 mol per L. Conversely, the apparent level of circulating surface protein in samples containing more than 10^7 IU per mL was similar to samples with the lowest DNA copy number.

A second group of donors was first tested before donation in 2002 to 2003 with a rapid test (Determine HBsAg, Abbott, Delkenheim, Germany) with a 1 ng per mL limit of detection. Negative donations were then tested by EIA (HBsAg Murex/Abbott) without dilution and found to be reactive. All samples were negative for the presence of anti-HBs. When tested by Q-PCR for the presence of HBV DNA, 53 samples were positive. In 45 cases, S/CO was between 1 and a maximum of 10. In that zone, S/CO was

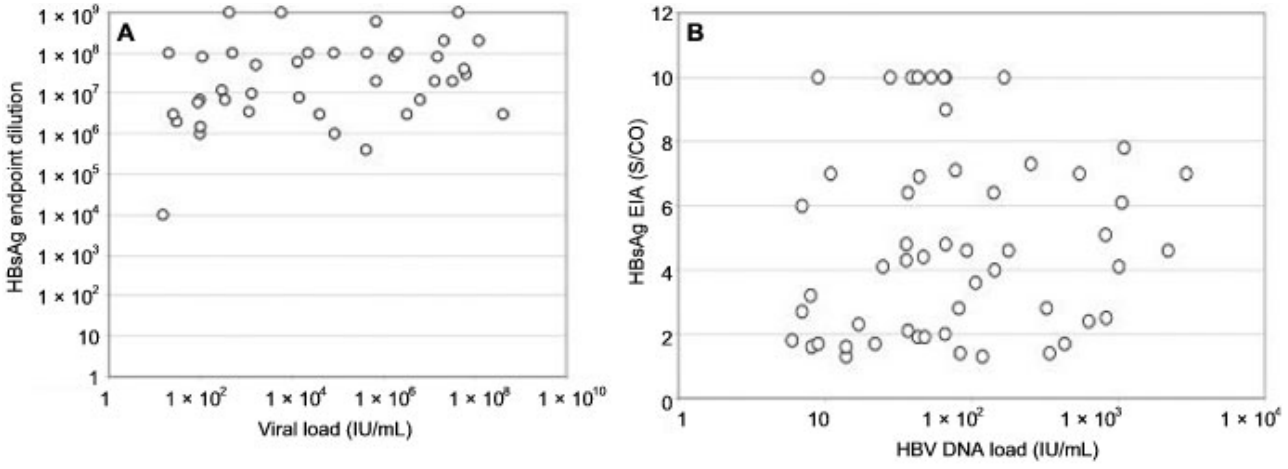

Fig. 1. (A) HBV DNA was quantified by real-time PCR as described in Allain et al.[2] Results are expressed in IU per mL. One IU per mL corresponds to approximately 5 copies per mL. All donors had ALT levels of greater than 60 IU per L, and samples were tested before any antiviral treatment was undertaken. (B) Blood donations did not react with a HBsAg rapid test (sensitivity, 1 ng/mL) used before donation did react with an EIA (sensitivity, 0.2 ng/mL). A majority of samples had a sample to cutoff ratio below maximum and a viral load below 100 IU per mL.

expected to reflect HBsAg concentration. Figure 1B shows the absence of correlation between HBsAg S/CO and HBV DNA load. Nearly all samples contained less than 1000 IU per mL HBV DNA, and 33 (62%) contained less than 100 IU per mL or approximately 500 copies per mL. Five samples (9.4%) were anti-HBc–negative.

Both sets of data presented concur with the results of Kuhns and coworkers[1] showing that, in chronic HBV infection, the apparent concentration of surface antigen in plasma does not correlate with the level of circulating viral DNA. This might reflect the considerable difference between the release of viral structural proteins and the formation of full virions released in the circulation. Nonencapsidated viral DNA tends to be rapidly destroyed whereas, in the absence of anti-HBs, surface antigen produced by either infected cells or integrated viral genome may remain in circulation for prolonged periods of time.

The gap in apparent HBsAg concentration between the first and second set of data is difficult to explain. Thirteen samples in the first set had a viral load below 1000 IU per mL, similar to what was found in the second set. Yet, S/CO below the maximum absorbance level was found after dilution of more than 10^4 in the first set and undiluted in the second, with the same EIA. Although the population of donors with liver disease tended to be older than those in set 2, and those in the first set with viral load below 1×10^4 IU per mL also tended to be older (median, 23 years vs. 28 years), neither difference was significant. It is possible that donors with liver disease have more integrated viral DNA producing HBsAg but no virions. The dual origin of HBsAg (viral replication and production from integrated DNA) might explain the presence of rare cases (3% in Kuhns et al.[1] and 2% in Allain et al.[2]) of detectable HBsAg without detectable HBV DNA. In such cases, a very low production of potentially infectious virus cannot be excluded. Conversely, the presence of occult HBV infection (HBV DNA–positive, HBsAg-negative) might not be rare in some populations.[3,4] As a result, blood safety might continue to benefit from sensitive detection methods of both HBsAg EIA and HBV DNA.

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Yield of hepatitis C virus nucleic acid testing among antibody-reactive or confirmed-positive samples

In contrast to acute hepatitis B virus infections in adults, of which only 1 to 3 percent become chronic, acute hepatitis C virus (HCV) infections progress to chronicity in a high proportion of cases, with reported rates of persistent viremia of up to 85 percent.[1,2] Spontaneous HCV clearance has been correlated with age at infection, race-ethnicity, HLA type, coinfection by other viruses, and immunosuppression.[3-5] Rates of HCV viremia detected by nucleic acid testing (NAT) have ranged from 74 to 86 percent in large seropositive populations, such as anti-HCV-reactive donors.[6-10] HCV NAT rates, however, may become lower with increased use of NAT and the introduction of more sensitive third-generation antibody assays.

In a recent article on NAT yield in German blood donors, Roth and coworkers[11] reported HCV viremia rates for 462 anti-HCV–positive donations confirmed by a second-generation semiautomated HCV antibody assay (Matrix, Abbott Laboratories, Delkenheim, Germany; before summer 2001) and antibody-reactive results confirmed by an HCV line immunoassay (Inno-Lia HCV antibody III update, Innogenetics, Gent, Belgium; after summer 2001). These seropositive samples had been tested by HCV NAT in pools containing up to 96 donor samples, and if RNA-negative, by single-sample NAT. Of 268 donor samples from the German Red Cross Institute Frankfurt, 187 (69.8%) tested RNA-positive, whereas only 84 (43.3%) of 194 samples from the Bavarian Red Cross tested positive by NAT. The surprisingly low rate of viremia observed in the HCV seropositive donors from the Bavarian Red Cross (relative to other donor populations in Germany and elsewhere) could not be explained by known correlates of clearance, which led to this investigation.

To confirm the RNA and antibody status of blood donor samples that had been classified as NAT-negative and HCV-seropositive in the previously published report, 110 of the original Bavarian donor samples and 5 of the original donor samples from Frankfurt were reanalyzed by two independent laboratories (Blood Systems Research Institute, San Francisco, CA, in collaboration with Chiron Corp., Emeryville, CA; and the German Red Cross Institute Frankfurt). The working plan and results from both laboratories are summarized in Fig. 1. All samples tested negative for the presence of HCV RNA (TMA Procleix Ultrio [Chiron Corp.] in San Francisco and in-house single-sample polymerase chain reaction [PCR] in Frankfurt), confirming the sensitivity of the original NAT screening system. Antibody-positive samples were confirmed by a third-generation HCV recombinant immunoblot assay (RIBA) (US) and by Inno-Lia HCV antibody III update (Germany). Whereas all five original samples from Frankfurt were confirmed HCV antibody–positive in both laboratories, only 9 of 51 Bavarian samples could be confirmed for the presence of HCV antibody with RIBA and 16 of 43 samples could be confirmed with Inno-Lia HCV antibody III update. Based on these confirmed HCV antibody results, the recalculated rates of viremia by minipool and single-sample PCR among the HCV antibody confirmed-positive samples were 187 of 268 (69.8%) and 84 of 109 (77%) for the Frankfurt and Bavarian Red Cross Centers, respectively. Therefore, there was no significant difference between the rates of viremia in these donor populations (chi-square test; $p = 0.19$), in accordance with other results in the literature.

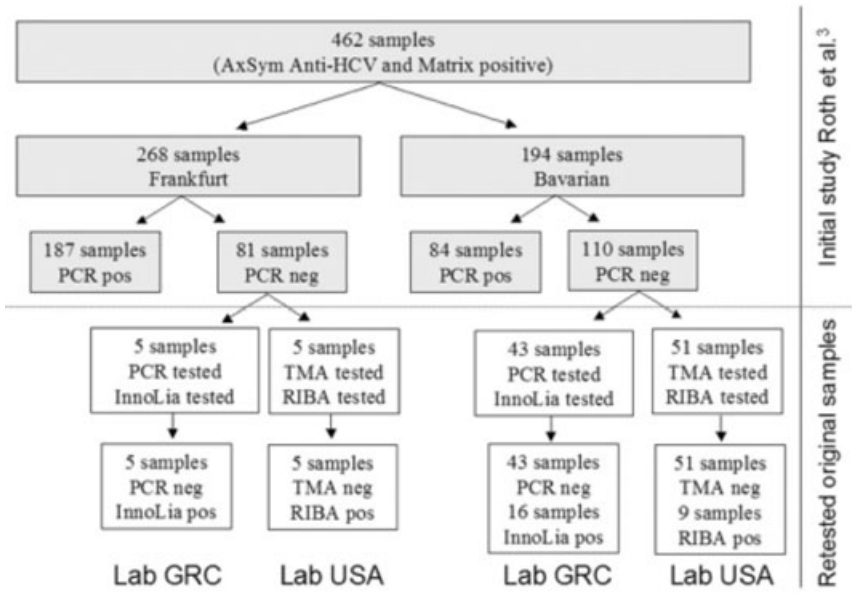

Fig. 1. Repeat analysis of 115 samples that had been initially confirmed HCV antibody–positive: 56 samples were analyzed in San Francisco by NAT TMA Procleix Ultrio (Chiron Corp.), anti-HCV Ortho ECi, anti-HCV Ortho third-generation enzyme immunoassay, and HCV third-generation RIBA. Forty-eight samples were retested in Frankfurt using in-house single-donor PCR, anti-HCV AxSym (Abbott Laboratories), and HCV Inno-Lia HCV antibody III update (Innogenetics). neg = negative; pos = positive.

Thus, the explanation for the low rate of reported viremia among the Bavarian donors was the inclusion of false-positive donor samples in the denominator of confirmed-positive donors, rather than the sensitivity of the NAT screening assay or unique demographic or epidemiologic factors. One explanation for these results could be the use of the less specific Matrix assay instead of RIBA or Inno-Lia HCV antibody III update in the initial study for confirmation of HCV antibody–reactive samples. In each institute (Bavarian Red Cross and German Red Cross Institute Frankfurt), 50 Inno-Lia HCV antibody III update–positive donations were additionally analyzed by NAT between 2001 and 2004. The Bavarian samples and the Frankfurt samples were 62 and 70 percent NAT positive, respectively. The recent data from both institutes is therefore in accordance with the literature and our retest results and indicate no significant difference between rates of HCV viremia among seropositive donors from either region. It is not possible, retrospectively, to determine the exact source of technical problems with the application of the second-generation Matrix HCV test at the Bavarian Red Cross before 2001.

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Re: Toward an understanding of transfusion-related acute lung injury: statement of a consensus panel

The recently published consensus statement on understanding transfusion-related acute lung injury (TRALI)[1] contains several errors pertaining to the work of our group (authors' reference 21).[2] Suspecting that the errors might reflect an incorrect bibliographical citation, we searched computerized databases, but cannot find an explanation. Virtually all of the citations to our work in this consensus statement are incorrect, as follows:

- "Rat lungs were ventilated, perfused . . . ." We did not use rat but rabbit lungs.
- ". . . and subsequently monoclonal anti-HNA-2a . . . was added to the perfusate . . . ." We did not use monoclonal, but polyclonal, alloantibodies from a blood donor, implicated in TRALI.
- (Same as above) The specificity of the antibody, added to the perfusate, was anti-HNA-3a or in former terminology anti-5b, not anti-HNA-2a.
- "The response was blunted when less than 30 percent HNA-2a-positive neutrophils were used." We did not find such a threshold of 30 percent and did not mention it.
- "The onset of these events was accelerated by the addition of the neutrophil activator formyl-methionyl-leucyl-phenylalanine." We did not use this activator.
- "Lung histology showed pulmonary edema and neutrophil accumulation." We did not perform histology; we just evaluated cell counts and microscopic smears.

Erroneous citations in this widely read consensus statement will, we are concerned, lead to misunderstandings with regard to our past, present, and future research in this field.[3-8] They should be rectified.

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The above letter was sent to Kleinman et al.: Drs Davenport and Kleinman offered the following reply.

We thank Neppert and v. Witzelben-Schürholz for their comments about our incorrect citation of their work and extend our sincere apologies to them and their colleagues. The data in our report attributed to Seeger and colleagues (our reference 21) should have been attributed to Bux. Bux presented these data on their ex vivo rat lung model at the consensus conference and have published these data in abstract form.[1]

Although the data were not presented at this conference, Neppert and colleagues previously published important pioneering work on an ex vivo model of transfusion-related acute lung injury (TRALI).[2] As pointed out in their letter, this model used isolated rabbit lungs that were perfused with human neutrophils, plasma containing anti-5b, and fresh rabbit plasma as a complement source. Appropriate control experiments were also conducted.

We would like to emphasize that apart from this incorrect citation, the consensus statement as published in **TRANSFUSION** accurately reflects the proceedings of the conference and the consensus of the full panel.[3]

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Naturally occurring anti-Jk^a

We report a case of "naturally occurring" anti-Jk^a in a 7-month-old boy with orchitis, epididymitis, and an *Escherichia coli* urinary tract infection.

The boy was born via cesarean section after 38 weeks of his mother's first and uneventful pregnancy. She had no history of transfusion. Physical examination was unremarkable, except for a tender mass in the left scrotum, which was consistent with orchitis or epididymitis by color Doppler examination. The complete blood cell count was normal. Culture of a midstream urine sample grew *E. coli*, more than 10^5 colony-forming units per mm^3. His red blood cells (RBCs) typed as group O, D+. An antibody detection test was positive and anti-Jk^a was identified by gel test using a low-ionic-strength saline (LISS)-Coombs card (DiaMed AG, Cressier, Morat, Switzerland). An antibody detect test was negative when repeated using a NaCl-enzyme card (DiaMed AG, Cressier). Anti-Jk^a activity was not reduced by dithiothreitol treatment, suggesting that the antibody was mostly immunoglobulin G (IgG). We did not perform a monocyte monolayer or other in vitro assay to evaluate the potential clinical significance of the antibody. The patient's and his mother's RBCs typed as Jk(a–b+). Both had negative results for direct antiglobulin tests and anti-nuclear antibody tests. The mother's antibody detection test was negative.

These serologic findings were unchanged on four subsequent serum samples collected during an 8-day hospitalization. An antibody detection test was negative when repeated 6 months after discharge.

We are aware of only one other case report of naturally occurring anti-Jk^a.[1] In that report, anti-Jk^a was detected by strong serologic reactivity in a solid-phase RBC adherence (SPRCA) assay, but not by indirect antiglobulin test tube method in LISS or polyethylene glycol or with papain- or ficin-treated RBCs. In contrast, anti-Jk^a in our patient had strong reactivity in the gel test, which we consider to be less sensitive than SPRCA. We are able to exclude primary alloimmunization because of maternal-fetal hemorrhage, because the patient's and his mother's RBC phenotypes were Jk(a–). Primary alloimmunization via maternal-fetal hemorrhage could not be excluded in the first report of naturally occurring anti-Jk^a, because the mother's RBCs were Jk(a+).[1]

Perhaps the most interesting finding in our study is the *E. coli* genitourinary infection. Several studies have described an association between blood group antigens and microbial infections,[2-5] although there appears to be only one report associating infection and Kidd blood group antigens.[5] That report described transient auto-anti-Jk^b in a patient with a proteus urinary tract infection.

Typically, naturally occurring antibodies are immunoglobulin M and, uncommonly, IgG as in our patient.

The basis for this phenomenon is not known. In the absence of information concerning the clinical significance of naturally occurring anti-Jk^a, in general, and for our patient's anti-Jk^a, specifically, we have recommended that Jk(a–) RBCs be selected, if our patient requires a future transfusion.

Although anti-Jk^a is detected rarely in the absence of exposure allogeneic Jk(a+) RBCs, we suggest that anti-Jk^a be added to future listings of blood group antibodies that may be "naturally occurring."

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SUBMISSION OF LETTERS

Instructions for submission of letters can be found in the Detailed Instructions for Authors published on pages 128 to 133 of the January issue. Submit letters to:

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