CMV DNA in blood donors with IgM and IgG CMV antibodies

Strategies for preventing posttransfusion CMV infections include testing for CMV antibodies, CMV DNA, and/or WBC reduction of blood components.[1,2] Early studies with molecular methods produced conflicting results. Nevertheless, recent investigations show that improved CMV PCR assays do not necessarily increase detection of CMV DNA in healthy CMV-seronegative blood donors.[3,4] We confirm these findings in a study of 244 plasma and WBC fractions and 176 serum samples from 420 donors in the Vienna, Austria, region (384 randomly selected and 36 samples selected for IgM anti-CMV reactivity).

We tested for IgM and IgG CMV antibodies in serum or plasma with two ELISAs (Enzygnost anti-CMV IgM and Enzygnost anti-CMV IgG, Dade Behring, Marburg, Germany). Results were interpreted to be positive if the corrected absorbance values ($\Delta A = [A_{antigen} - A_{control\ antigen}] \times$ correction factor) were greater than the cutoff of 0.2 OD, negative if the absorbance values were less than the cutoff of 0.1 OD, and equivocal if the absorbance values were between 0.1 and 0.2 OD in the first test and in retesting. CMV PCR screening was performed on serum, plasma, and WBC samples with a 5′-nuclease real-time PCR assay, which targets the polymerase gene region of the virus. PCR-reactive samples were retested and confirmed with a second real-time assay amplifying a sequence stretch in the major immediate early region. In serial dilutions of the quantitated plasma sample VQC 1999/6, the 95 percent cutoff value of the screening assay had been estimated to be four copies per PCR (192 copies/mL).

CMV DNA was reproducibly detected and confirmed in the WBC fraction from one donor and a serum sample from another donor. CMV DNA was not detected in the plasma or whole blood corresponding to the CMV DNA-positive WBCs. We interpret these results to indicate that this sample was most probably from a latently infected individual. The viral loads in these samples were approximately 10 times lower (approx., 100 copies/mL) than the positive control for the assay (1000 copies/mL).

The above findings correlate well with those of Roback and colleagues.[4] In their study, 10 to 99 CMV geq per 250,000 WBCs were detectable in 2 of 1000 whole blood samples. Both samples were reactive for anti-CMV but there was no differentiation between IgM and IgG reactivity. We also found our positive DNA results only in seropositive samples, in the strict sense restricted to IgG-positive and either IgM-equivocal or IgM-negative seroreactivity (Table 1).

It is unclear whether CMV DNA detected in the serum sample showing an IgM-equivocal result and IgG antibody positivity corresponds to a late phase of primary CMV infection, a reinfection, or a reactivation of a latent infection. Because Drew and colleagues[3] did not detect CMV DNA in 488 plasma samples from 60 persistently seropositive individuals, but observed plasma CMV DNA in a small percentage of seroconverting blood donors, the first explanation seems to be more probable.

TABLE 1. Results of CMV PCR testing, correlated with CMV antibody reactivity

CMV antibody reactions	Number	CMV-DNA-positive samples
Random samples (n = 384)		
IgM-equivocal,* IgG-positive	16	One serum sample
IgM-negative, IgG-positive	185	One WBC sample
IgM-negative, IgG-equivocal*	5	None
IgM-negative, IgG-negative	178	None
IgM-reactive samples (n = 36)		
IgM-positive, IgG-positive	9	None
IgM-equivocal,* IgG-positive	27	None

* The test result of a sample was classed as "equivocal" if it was between 0.1 and 0.2 OD in the first test and in retesting.

In conclusion, our results confirm that CMV DNA is rarely detected in asymptomatic CMV-seropositive blood donors and is absent in CMV-seronegative individuals. If CMV NAT is to have a role in donor screening, it will most probably be limited to the detection of CMV-seronegative window-phase donations.

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PLT-associated immunoglobulin increases during PLT storage

In the US and Europe, there is increasing interest in extending the permissible storage time for PLTs. In two recent studies, Dumont and coworkers[1,2] compared the viability and clinical efficacy of apheresis PLT concentrates stored for 5 and 7 days, respectively. Not surprisingly, 7-day storage was accompanied by further decreases in pH and the hypotonic shock response and a two- to sevenfold increase in P-selectin expression. Transfusion of 7-day-old autologous PLT concentrates was accompanied by additional decreases in PLT recovery (14%) and survival (17%), possibly owing to increased PLT activation.[2]

We have also examined the effect of increasing storage time on PLT concentrates, particularly with regard to immunologic markers suggestive of PLT senescence. In RBCs and PLTs, there is a relationship between in vivo senescence and the binding of naturally occurring autoantibodies, which recognize neoantigens generated by progressive degradation of the membrane glycocalyx.[3,4] Because PLTs are stored in autologous plasma, which contains a variety of proteases, glycosidases, and naturally occurring autoantibodies, PLT concentrates may undergo a similar process during in vitro storage. To examine the latter, whole-blood-derived PLT concentrates ($n = 7$) were assayed for pH and PLT-associated IgG and IgM (PAIgG, PAIgM) by flow cytometry on Days 1 through 8 and Day 14 of storage. Each PLT sample was tested in triplicate and recorded as the MFI per sample. PAIgG and PAIgM were compared relative to pH and day of storage with Pearson-product moment correlation and computer software (KaleidaGraph, Synergy Software, Reading, PA). A p value of less than 0.05 was considered significant.

As shown in Figs. 1A and 1C, both PAIgG and PAIgM increased over time, rising 2- and 3-fold over baseline by Days 5 and 7, respectively. Increased PAIgG and PAIgM were significantly correlated with both storage time ($r = -0.56$, PAIgG; $r = -0.58$, PAIgM; $p < 0.005$) and pH ($r = 0.70$, PAIgG; $r = 0.70$, PAIgM; $p < 0.05$), which fell from pH 7.44 to pH 7.19 by Day 7 of storage (Fig. 1E). By Day 14, there was a significant increase in H+ concentration (pH 6.46), accompanied by a 14- to 15-fold rise in PAIgG (MFI, 65.5; $p < 0.005$) and PAIgM (MFI, 73.6; $p < 0.005$). Using a human anti-HPA1 as a standard, it is estimated that Day 7 PLTs averaged nearly 4000 IgG molecules per PLT, which is equivalent to that reported in autoimmune thrombocytopenia.[5] By Day 14 of storage, these values rose to over 16,000 IgG and 18,000 IgM molecules per PLT.

Our results suggest that in vitro PLT storage is associated with increasing autoantibody binding, reaching levels sufficient to mediate immune clearance by the reticuloendothelial system. Transfusion of older, antibody-coated PLTs may contribute to the progressive decrease in posttransfusion recovery and survival reported by Dumont and others.[2] This increase in PAIgG and PAIgM should not be surprising, given the short in vivo life span of PLTs (9-10 days). If stored PLTs age at a rate and fashion comparable to that in vivo, potentially 50 percent of PLTs have reached immunologic "senescence" by Day 5, and 75 percent by Day 7. Although we did not identify the nature of the autoantigens generated on PLTs, it is likely that they mimic those observed on aging RBCs. Like RBCs, PLTs possess a number of sialoglycoconjugates that, upon desialylation, can give rise to several autoantigens recognized by naturally occurring, "senescent" antibodies (T, Tn, anti-Gal).[3,4] In animals, there is an inverse correlation among PAIgG, membrane sialic acid, and PLT survival.[4] It may also account for the inverse relationship between pH, PAIgG, and PAIgM: sialic acid is degraded at acid pH. Autoantibodies may also recognize confirmation-dependent neoantigens on activated PLTs.

In summary, the PLT storage lesion is a complex, multifaceted problem with significant implications for extending PLT storage. In addition to PLT activation and falling pH, PLTs continue to undergo aging and senescence, as evidenced by apoptosis-like proteolysis and increasing autoantibody binding. New research into alternative storage media may alleviate several of the mechanisms associated with PLT storage, including the increase in PAIgG and PAIgM observed in our study.

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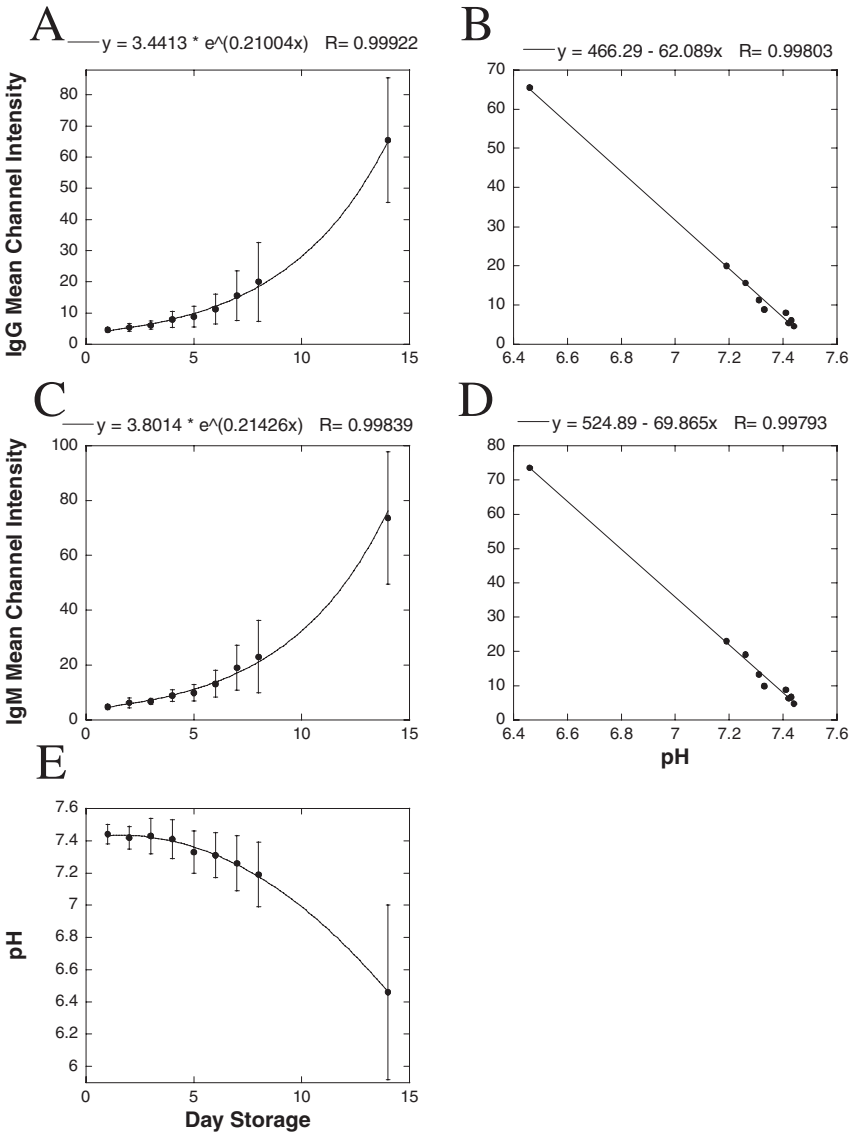

Fig. 1. PLT-associated immunoglobulin increases with increasing storage and decreasing pH. Shown are the effects of increasing storage time (days) on PAIgG (A, mean ± SD, n = 21), PAIgM (C), and pH (E). PAIgG and PAIgM were determined by flow cytometry and recorded as MFI. The CV for both IgG and IgM was approximately 20 percent for Days 1 through 5 and 30 to 40 percent for Days 6 through 14. (B, D) Correlation between plasma pH during storage and PAIgG (B) and PAIgM (D).

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Genotyping Dombrock alleles in Portuguese blood donors by real-time PCR

A recent review of the Dombrock blood group system by Reid[1] cites PCR-RFLP and allele-specific PCR as methods that can be used to differentiate among Dombrock alleles.[1] In our experience, these methods are time-consuming and cumbersome. As an alternative, we developed a method to genotype blood group alleles by real-time fluorescence PCR[2,3] and now report the results of genotyping 100 Caucasian Portuguese blood donors for Dombrock *DO1* and *DO2* alleles with this method.

DNA from 100 blood donor samples was extracted with a DNA isolation kit (Magna Pure LC, Roche, Mannheim, Germany). Molecular analysis was conducted by a cycler system for the detection of amplification products (LightCycler, Roche Molecular Systems, Somerville, NJ), with hybridization probes. Primers (nucleotides [nt] 754-774 and 950-928) and probes (nt 908-889 and 887-855) were designed by TIB MOLBIOL (Berlin, Germany). Amplification and detection methods included initial denaturation at 95°C for 120 seconds, followed by 45 cycles, with denaturation at 95°C for 0 seconds, annealing at 65°C for 10 seconds, and extension at 72°C for 10 seconds. The melting point for *DO1* was 58°C, and for *DO2,* 65°C. In different runs, the positions and distances of melting peaks for the same allele were identical, varying by less than 1°C.

Genotype frequencies for *DO1*/*DO1* (0.20), *DO1*/*DO2* (0.46), and *DO2*/*DO2* (0.34) and for the allele *DO1* (0.43) in our Portuguese population were similar to frequencies reported for the corresponding phenotypes in Caucasian

populations, but different from those reported for non-Caucasian populations.[1] Real-time fluorescence PCR is robust, has few manual steps, and is suitable for large-scale blood group genotyping.

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The above letter was sent to Dr Reid, who offered the following reply.

It is rewarding to read that genotyping is being used to establish the frequency of *DOA* and *DOB* alleles in a specific population. Owing to the paucity of serologic reagents, this information was not possible to obtain by hemagglutination. It is important to establish such data as DNA analysis becomes more widespread.

Araujo and associates state that in my review of the Dombrock blood group system,[1] I refer only to PCR-RFLP and allele-specific-PCR (AS-PCR). While this is true, I merely noted published methods and did not intend to imply that these were the only DNA-based methods that could be used. Once a gene has been cloned and sequenced and the molecular bases have been associated with antigens, it is probable that any DNA-based method can be applied. "Real-time" PCR with a light cycler is one method. Other methods include mass spectrometry and the pyrosequencer. PCR-RFLP and AS-PCR have the advantage of not requiring dedicated (and often costly) equipment. Also, for the record, the PCR-RFLP assay described by us[2] and a subsequent modification with *Bse*RI[3] in place of *Eam*1105I were designed to give a unique banding pattern for each allele, thereby avoiding false results as a result of incomplete digestion.

When using genetic information, it is appropriate to give credit to those who established the molecular basis associated with the alleles. In the case of *DOA* and *DOB,* the work of Gubin and coworkers[4] is fundamental to the development of assays such as described by Araujo and colleagues. It would seem appropriate to reference this work or provide the GenBank accession number that pertains to establishing a new assay.

It is pertinent to note that all three nucleotide changes in the coding region of *DO* are indeed "relevant" to the alleles, although only 793A>G is predicted to change an amino acid. Interestingly, both *HY* alleles have the nucleotide associated with *DOA* at position 378 and nucleotides associated with *DOB* at positions 624 and 793. In contrast, the *JO* allele has the nucleotides associated with *DOB* at position 378 and nucleotides associated with *DOA* at positions 624 and 793.

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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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