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Reply

To the Editor—We appreciate the comments of Volpi et al. [1] about our study [2]. Our results clearly showed an enormous variation in antibody levels against human cytomegalovirus (HCMV) in individual patients after bone marrow transplantation. Eight- to 10-fold changes in titer within a 2-week period were not uncommon, regardless of whether viral replication or HCMV disease was detected. Thus, we feel it is essential to evaluate the kinetics of antibody response in the individual patient. Statistical analyses of a few time points after transplantation or grouping of patients, especially when there are few samples or patients, are not meaningful, since the antibody response after transplantation is so different between patients. Volpi et al. consider a neutralizing antibody response greater than the 90th percentile of asymptomatic patients to be high and postulate a lack of correlation between high neutralizing antibody titers and clinical outcome of the infection.

It would be important to know (1) what posttransplantation time point(s) was used to calculate those titers, (2) the range of neutralizing antibody titers in these patients, and (3) the variation of the assay. Volpi et al. [1] also considered patients showing a 4-fold increase in neutralizing antibody titer to be serologic responders and concluded that serologic response is not important for the clinical outcome of the infection. As mentioned above, we have seen much higher titer fluctuations in individual asymptomatic patients in the absence of any sign of viral replication (determined by polymerase chain reaction and/or antigenemia). Again, it would be important to see the changes in neutralizing titers over time in the individual person.

We agree with our colleagues that HCMV strain variations might have a significant effect on the evaluation of neutralizing antibody titers in human sera [3] and that more studies are needed to establish the role of anti-HCMV antibodies for the clinical course of the disease in transplant recipients.

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TT Virus—Part of the Normal Human Flora?

To the Editor—Since the original description of TT virus (TTV) [1], intensive efforts have been made to determine the clinical significance of TTV infection, such as a possible etiologic role in posttransfusion, fulminant, and idiopathic chronic hepatitis. These studies have been hampered by difficulties in diagnosing current or past infection with TTV; for example, titers of viremia are low, and it is unclear whether currently used polymerase chain reaction (PCR)–based methods for screening are adequate to detect all genetic variants of TTV. Problems with current PCR-based methods for TTV detection were illustrated in the recent publication by Desai et al. [2], who reported a high frequency of discrepant results between different sets of N22-specific primers. The authors suggested that multiple primer pairs must be used to maximize the sensitivity of the PCR. Takahashi et al. [3] developed a PCR for TTV using

Table 1. Detection of TT virus in different pediatric age groups and in adults in different countries.

Study group	No. positive/no. tested	Prevalence (%)
Pediatric population		
0–<3 months	0/7	0
3–<12 months	6/8	75
1–5 years	34/44	77
6–10 years	37/49	76
11–18 years	28/40	70
Unpaid blood donors		
Scotland	46/100	46
The Netherlands	69/96	72
Finland	73/100	73
Singapore	41/42	98
Saudi Arabia	48/48	100

primers from the conserved 5′ noncoding region (NCR) and reported a remarkable 92% prevalence of viremia in the general Japanese population. Since a 23% prevalence of TTV viremia was reported in Japan when N22-based primers were used [4], the observation of a substantially greater frequency of infection when 5′ NCR primers are used suggests that the high degree of genetic variability of TTV was preventing its detection in the majority of the population.

To investigate these findings in other populations and to determine the time of acquisition of TTV infection, we screened anonymous samples from infants, children, and adults by using the 5′ NCR primers [3]. DNA was extracted from 100 μL of plasma [5] and amplified over 40 cycles using Hotstar Taq (Qiagen, West Sussex, UK).

All samples collected from infants before age 3 months were negative for TTV, whereas prevalences of detectable viremia ranged from 46% to 77% in older infants, children, and Scottish adults (table 1). Even higher frequencies of TTV infection (72%–100%) were detected in other European and non-European blood donor populations. The absence of detectable TTV infection before age 3 months indicates that infection was unlikely to have been acquired in utero and suggests perinatal or postnatal environmental sources of infection.

The frequencies of TTV infection detected with the 5′ NCR primers are much higher than those detected with the original heminested primers (1.9%–4% in Scottish donors [5], 17% in Finland, and 19% in Saudi Arabia) and suggest that the majority of infections were caused by variants highly divergent in sequence from the original isolate [4]. Indeed, even the new PCR method may underestimate the actual frequency of TTV infection, as it is restricted to one round of amplification, while the primers were based on regions of the genome whose genetic variability remains incompletely characterized. Even higher frequencies or universal TTV infection may be found in human populations when methods for TTV detection are optimized further.

These findings negate the value of current attempts to link TTV infection with specific clinical outcomes, such as posttransfusion hepatitis or other forms of liver disease. It is possible that certain genetic variants of TTV, such as those detected with the original N22-based primers, may have specific disease associations. However, the finding of very high frequencies of infection of persons in tropical countries when these primers are used [6] makes this less likely. We regard TTV as an example of a highly successful and widely distributed virus capable of establishing a commensal relationship with its host.

In the future, the widespread use of subtractive PCR-based methods for novel virus detection is likely to lead to the discovery of further human viruses. As exemplified by the investigations of TTV, the difficulty will be to determine true frequencies of infection and disease associations. However, from an evolutionary biology perspective, the new knowledge of TTV provides a fascinating new paradigm for virus persistence and the evolution of host/virus relationships.

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