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NOTES

Relationships between Total Plasma Load of Torquetenovirus (TTV) and TTV Genogroups Carried

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In 239 torquetenovirus-positive people, multiple-genogroup infections were common and associated with higher viral loads than would be expected from simple additive effects. The latter observation was restricted to the infections which included both genogroups 1 and 3, pointing to the possible existence of some kind of infection facilitation between these genogroups.

Torquetenovirus (TTV), formerly known as TT virus, was discovered in 1997 by Japanese workers in the blood of a patient with cryptogenetic hepatitis and has since been shown to sustain long-term, possibly permanent viremia in a surprisingly high proportion (around 80%) of the general population worldwide (1–3, 5, 12, 13). It is also clearly documented that the loads of TTV (viral load [VL]) in plasma may differ extensively among individual hosts (15). Due to its high genetic heterogeneity, TTV is currently subdivided into five highly divergent genogroups, designated 1 to 5 (4, 14). Although multiple-genogroup TTV infections are quite frequent (1, 6, 7), there have been no reported studies on the possible relationships between the number of genogroups carried and the total VL present in an individual.

In the course of our studies, we noted that individuals with high VLs tended to yield more TTV genogroups than those with low VLs. Thus, in preliminary work, we examined whether this might be due to the fact that high VLs permitted detection of TTV genogroups which escaped detection in low-VL plasma because they represented a small fraction of the total VL. The experiments did not support this explanation. In particular, the processing of five low-VL plasma specimens that had yielded one or two TTV genogroups in such a way as to augment 10- and 100-fold the amount of DNA examined in the typing assays had little or no impact on the number of genogroups detected. Similarly, when five specimens positive for four genogroups and with VLs between 6.1 and 6.8 $\log_{10}$ copies per ml were diluted 100-fold, i.e., to a point where the VLs were in the range that is associated mostly with single-genogroup infections, they still yielded three to four TTV genogroups (data not shown).

Such failure to provide an easy explanation for the observation prompted a systematic look at the issue. To this purpose, we assessed the presence, load, and genogroup(s) of TTV in plasma samples from 300 people. The methods have been previously reported (8, 9, 15). Briefly, extracted DNA was assayed for TTV presence and load with a universal TaqMan real-time PCR having the potential to detect and quantitate all hitherto-recognized genetic forms of the virus (8, 9). TTV-positive DNA extracts were then typed with five distinct genogroup-specific PCRs, as described previously (8, 9), using the primer sets shown in Table 1.

TTV was detected in 267 people (89%) and successfully typed in 239. Most TTV-positives carried two distinct genogroups, followed by one, three, and four. TTV genogroups 1 and 3 were the most prevalent, followed at a distance by genogroups 4 and 5, while genogroup 2 was rather infrequent (Table 2). The failure to type TTV in the remaining 10% of the virus-positives was probably due to the lower sensitivity of the typing PCRs relative to the universal PCR (4,000 versus 1,000 DNA copies per ml), since the mean VL for this group of virus positives was 4.1 ± 0.9 $\log_{10}$ copies/ml compared to 5.1 ± 1.1 $\log_{10}$ copies/ml for those whose virus could be typed. However, the possible presence of TTV forms unrecognized by the typing protocol used cannot be excluded.

We then stratified the VLs of the 239 typed subjects according to the number of TTV genogroups they harbored (Fig. 1). Interestingly, on a log scale, the correlation between average VLs and numbers of TTV genogroups carried was linear, indicating an exponential increase in VL with an increasing number of genogroups harbored. Indeed, the VL increases were invariably greater than what would be expected based on simple additive effects: in dual-, triple-, and quadruple-genogroup infections, the average VLs were 5.0, 5.5, and 6.3 $\log_{10}$ copies/ml rather than 4.7, 4.9, and 5.0 $\log_{10}$ copies/ml, as would be expected from two-, three-, and fourfold increases, respectively, over those in the single-genogroup infections. Furthermore, breaking down the subjects into age, gender, or disease groups showed similar trends in the distribution of VL sizes,

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TABLE 1. PCR primers used for TTV genogrouping

Genogroup	Primer	Sequence (5′-3′)[a]	Genome localization	Position (nt)[b]	PCR product length (bp)
1	G1-1	AACGARGACCTAGACCTITGTAGATA	ORF1	1021–1046	289
	TTV2	CAGTTAGTGGTGAGCCGAA	ORF1	1348–1366	
	G1-3	TTYAGACACCCARAIGTAGACTT	ORF1	1069–1091	
	G1-4	GTGAGCCGAACGGATATTG	ORF1	1339–1357	
2	G2-1	AATATGACMCCTTTGGAGGIGG	ORF1	945–966	260
	G2-2	TGAGCAGAACGGAAACCGCAAG	ORF1	1357–1378	
	G2-3	CTGGAGIAGATCGAACRTAGA	ORF1	1030–1050	
	PMV4	CTGTAAATAGAGTGGGGGG	ORF1	1271–1289	
3	G3-1a	AAYGACCAGCTAGACCTIGCCAGATA	ORF1	1058–1083	263
	G3-1b	AAYACTCAGCTAGACCTIGCYAGAT	ORF1	997–1021[c]	
	G3-2	TTWGTGGTGRGCIGAACGG	ORF1	1382–1400	
	G3-4	TGKGTGTACCAITTRTCTWCAA	ORF1	1299–1320	
4	G4-1	CCATTTTGTGCAGCCCG	UTR	103–119	295
	G4-2	CGGCGGACTCCACGGCAT	UTR	403–420	
	G4-3	AGCCCGCCAATTTCTGTT	UTR	114–131	
	G4-4	ACGGCATGAYTTTGTGTCCTG	UTR	389–409	
5	G5-1	CCAACTGCAAAGAAAAAACCACCT	UTR	305–328	271 bp[d]
	G5-2	CGCCTCCTTACTCTTCGTCGTC	UTR	701–722	
	G5-3	CGAGCACCTCTGGTACGAGTC	UTR	373–393	
	G5-4	GTCTGCGAAGTCTGCCACGGG	UTR	650–670	

[a] I, inosine; R, A or G; Y, C or T; M, A or C; W, A or T; K, G or T.
[b] Nucleotide (nt) positions are based on sequences of isolates AB017610, AF261761, AB028669, AB038624, and AB064606 for group 1-, 2-, 3-, 4-, and 5-specific PCRs, respectively, unless indicated otherwise.
[c] Nucleotide positions are based on the sequence of isolate AB050448.
[d] PCR products range between 271 and 306 bp depending on the presence of insertions or deletions.

depending on the number of genogroups carried, thus corroborating the observation (data not shown).

We then examined whether the observation described above was unspecific or involved some particular genogroup combination(s) (Fig. 2). Single-genogroup-infected subjects showed no significant differences in mean VL, regardless of whether they carried genogroup 1, 3, or 4 (no single genogroup 2 infections were present in our series, and single genogroup 5 infections were too few for statistical analysis), thus showing that at least these genogroups are not inherently different in the viremia levels they establish when present alone. In contrast, analysis of the three categories of dually infected people that could be compared, namely, those infected with genogroups 1 and 3, 1 and 4, and 3 and 4 (the sizes of the other categories were too small for meaningful comparisons) showed that, on average, the VL was 10-fold higher in those coinfected by genogroups 1 and 3 than that in the pooled carriers of the other two combinations ($P = 0.013$; Student's t test). In addition, comparing the mean VLs of all single- and dual-genogroup infections yielded a significant heterogeneity ($P < 0.001$; analysis of variance test), and Tukey's multiple-comparison test showed that coinfection by genogroups 1 and 3 resulted in the only true outlier ($P < 0.01$). With regard to triple infections, we could compare only the VLs of those in whom both genogroups 1 and 3 were present with all the others; again, the mean VL was 10-fold higher in the former than in the latter, albeit the difference was not statistically significant, probably due to the small sample size.

In summary, these findings (i) confirm that multiple-genogroup TTV infections are quite common; (ii) demonstrate that in single-genogroup-infected individuals the TTV genogroups that could be evaluated produce comparable levels of viremia; and (iii) exclude the existence of major impediments between TTV genogroups that might stem from receptor-mediated interference, cross-immunity, or other causes. In addition, the results reveal that subjects coinfected with TTV of genogroups

TABLE 2. Number and identity of the TTV genogroups detected in the 239 study subjects whose infections proved typeable[a]

Genogroup parameter	No. (%) of study subjects
No. of genogroups detected	
One	73 (30)
Two	81 (34)
Three	60 (25)
Four	25 (11)
Identity of the TTV detected	
Genogroup 1	167 (70)
Genogroup 2	6 (2)
Genogroup 3	175 (73)
Genogroup 4	116 (48)
Genogroup 5	51 (21)

[a] The subjects included 68 patients with hepatitis C, 53 patients with acute respiratory diseases, 67 patients with rheumatic and immune diseases, 22 patients with miscellaneous diseases, and 29 healthy controls. Number of males, 132; number of females, 107. Age ranged from 1 month to 82 years. All were human immunodeficiency virus negative. All subjects (parents in the case of children under 18 years of age) gave informed consent to be included in the study.

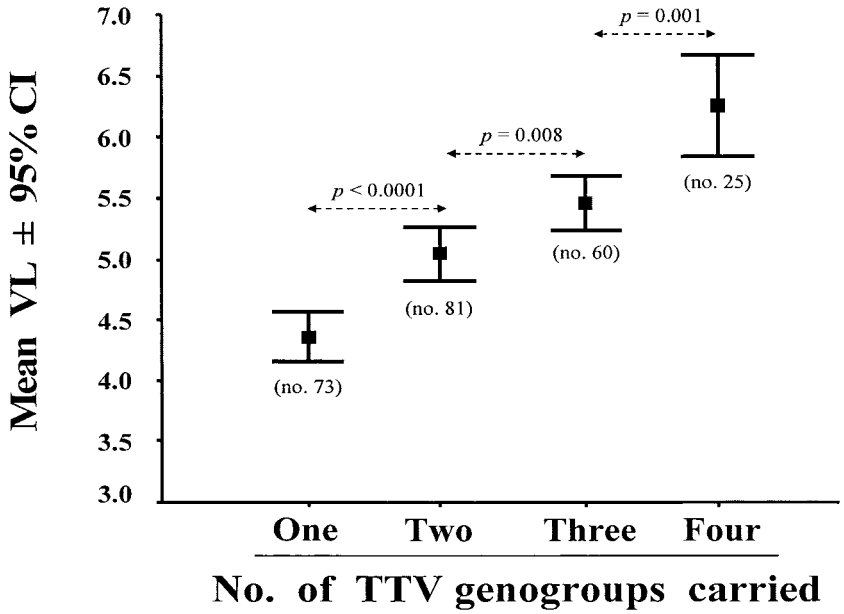

FIG. 1. VLs of the 239 subjects whose TTV infections proved typeable, stratified by the number of viral genogroups carried. VL is expressed as the mean $\log_{10}$ TTV DNA copies per ml of plasma ± confidence intervals (CI). The numbers in parentheses are numbers of patients in the respective groups. Statistical significance was calculated with the two-tailed, unpaired Student *t* test.

1 and 3 tend to have higher-than-expected VLs. A possible explanation for this last finding is the existence of some kind of infection facilitation between these two genogroups, which might result from several mechanisms, including help at the cell level by transactivation or complementation or at the organism level by immunomodulation (9–11, 16) or antibody-mediated endocytosis. Such facilitation might help to explain the larger prevalence worldwide of genogroups 1 and 3 relative to other TTV genogroups. An alternative explanation is that the individuals carrying both genogroups 1 and 3 have particularly high VLs because they can become infected with especially numerous strains of TTV, due to the fact that these two

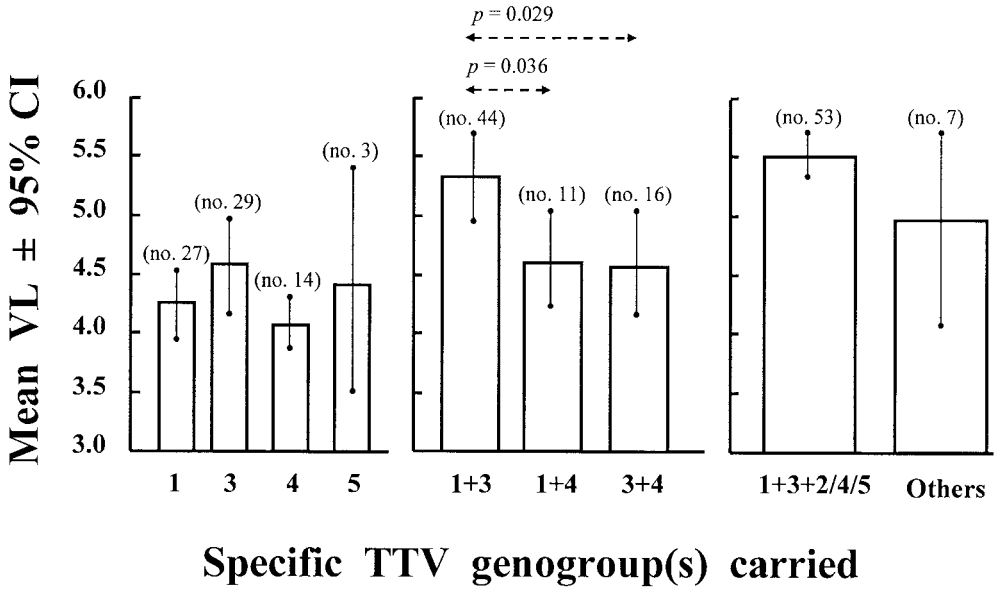

FIG. 2. VLs of the 239 subjects whose TTV infections proved typeable, stratified by the specific viral genogroup(s) carried. (Left panel) Single-genogroup infections; (central panel) dual-genogroup infections; (right panel) triple-genogroup infections. VL is expressed as described in the legend to Fig. 1. The numbers in parentheses are numbers of patients in the respective groups. Statistical comparisons were performed by the two-tailed, unpaired Student *t* test. Due to the small sample size, the group singly infected with genogroup 5 was excluded from analysis. The *P* values for significant differences are shown.

genogroups not only are the most prevalent but also contain large arrays of viral genetic forms, including genotypes and species (4, 6, 14). Distinguishing between these possibilities will require an exhaustive evaluation, by cloning and sequencing, of the entire genetic diversity of the TTV present in large numbers of single- and multiple-genogroup-infected subjects.

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