Lack of transmission of TT virus through immunoglobulins

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BACKGROUND: A high prevalence of TT virus (TTV) infection has been found in patients who received blood or blood components. Viral DNA was demonstrated in commercial preparations of FVIII and F IX, but very few data have been reported on immunoglobulins. The risk of TTV infection associated with intramuscular or IV immunoglobulin administration is unclear.

STUDY DESIGN AND METHODS: The prevalence of TTV infection in a group of patients undergoing lifelong therapy because of congenital immunodeficiency has been evaluated in a long term follow-up (median, 6 years). Seventeen patients with congenital immunodeficiency receiving monthly administration of IVIG were included in the study. TTV DNA was repeatedly evaluated by PCR in serum samples from each patient during the follow-up. Research of antibodies against TTV was not applicable, as the patients studied were unable to produce antibodies. The presence of TTV was also evaluated in 15 IVIG lots.

RESULTS: The total amount of immunoglobulin administered was 18,773 g. TTV infection was not found in any patients included in the study. None of the 15 immunoglobulin preparations analyzed was found positive for TTV DNA.

CONCLUSION: Despite the high prevalence of TTV in blood donors, commercial immunoglobulins are safe and unable to transmit TTV.

ABBREVIATIONS: TTV = TT virus; IMIG = intramuscular immunoglobulin.

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A novel DNA virus, TT virus (TTV), has been implicated as a cause of posttransfusion hepatitis.[1] The existence of several genotypes of the virus has been shown by studies,[2,3] and, as with HCV, multiple variants may be found in the same patients.[4,5] In all the variants, the noncoding region of the viral genome is the most highly conserved. On the other hand, the sequence of the coding region, which is more variable, is usually used to divide the virus into different genotypes. At present, more than 15 genotypes have been described.[6] Correlation between genotypes and the geographic source of the virus has not been shown.[4] A high prevalence of TTV infection has been found in patients who received blood or blood components.[7,8] Viral DNA was demonstrated in commercial preparations of FVIII and F IX.[8] Other parenterally transmitted viruses such as HCV, have been shown in intramuscular immunoglobulin (IMIG) or IVIG preparations,[9,10] and sporadic outbreaks of HCV infections occurred before 1994.[11]

Recently, the presence of TTV DNA was confirmed in IMIG batches,[12] but very few data have been reported on IVIG. Little is known about the risk of TTV infection in association with IMIG or IVIG administration. The aim of the present study was to investigate the prevalence of TTV infection in a group of patients undergoing lifelong therapy with monthly administration of IVIG because of congenital immunodeficiency.

MATERIALS AND METHODS

Patients

Seventeen patients (12 males, 5 females) with congenital immunodeficiency were included in the study. All the patients received monthly doses of IVIG (Endobulin, Baxter, Pisa, Italy; IgVENA, Sclavo-Chiron, Siena, Italy; or Venogamma, Alfa Wassermann, Pescara, Italy). Two patients (men, 29 and 30 years old) had received IMIG before IVIG became available. The median age of the patients at the time of the study was 50 months (range, 17 months–30 years). All the patients had been followed since they first received immunoglobulins. The median follow-up on the patients studied was 6 years (range, 12 months–25 years). In Table 1, the immunologic disease diagnosed, the age and sex of the patients, and the duration

of IVIG therapy are reported. For each patient, at least two serum samples were analyzed during the follow-up. All of the sera analyzed had been stored at –80°C and not thawed before testing for TTV DNA.

Detection of TTV sequences

Total DNA was extracted from 200 µL of serum by use of a blood kit (QIAamp, QIAGEN Ltd, Crawley, UK) and resuspended in 50 µL of elution buffer. Total DNA was also extracted from 200 µL of 15 samples of IVIG (2 archived and 3 current preparations for each of Endobulin, IgVENA, and Venogamma). Samples of IMIG were not available for testing.

TTV DNA was amplified by semi-nested PCR with TTV-specific primers derived from a conserved region of the published sequences.[13,14] Specifically, TTV DNA was amplified by PCR in a thermal cycler (2400, Perkin-Elmer, Emeryville, CA) by the following protocol: 35 cycles at 94°C for 30 seconds, at 58°C for 30 seconds, and at 72°C for 45 seconds and 1 cycle at 72°C for 7 minutes. The reaction conditions were 20 pmol of each primer (Primer A: sense, 5′-ACA GAC AGA GGA GAA GGC AAC ATG-3′, position 1900-1923 from ORF-1; Primer B: antisense, 5′-CTG GCA TTT TAC CAT TTC CAA AGT-3′, position 2185-2161) and 2.5 U of *Taq* polymerase (Polymed, Milan, Italy) in a 50-µL reaction volume. Five µL of the products of amplification was amplified in a second round of PCR by use of a semi-nested primer set (primer C: sense, 5′-GGC AAC ATG TTA TGG ATA GAC TGG-3′, position 1915-1938; antisense, the same Primer B) under the same conditions described above. The length of the amplicons obtained was 270 bp. Multiple positive and negative controls were included in each PCR assay. PCR products were analyzed in a 2-percent agarose gel electrophoresis with ethidium bromide staining.[7] For IVIG samples and for pooled sera that were negative for TTV, 10 µL of a positive serum sample was added and TTV testing was repeated to exclude the presence of possible Taq inhibitors. Research on antibodies against TTV was not applicable, as the patients studied were unable to produce immunoglobulins.

TABLE 1. Diagnosis of immunologic disease, age, sex, and duration of IVIG therapy in 17 patients with congenital immunodeficiency who received monthly IVIG doses

Patient	Diagnosis	Age (months)	Sex	Duration of IVIG therapy (months)
1	X-linked agammaglobulinemia	360	M	300 *
2	X-linked agammaglobulinemia	344	M	288 *
3	X-linked agammaglobulinemia	145	M	139
4	X-linked agammaglobulinemia	50	M	44
5	X-linked agammaglobulinemia	18	M	11
6	Severe combined immunodeficiency	74	M	68
7	Autosomal agammaglobulinemia	142	F	138
8	Autosomal agammaglobulinemia	127	F	111
9	Common variable immunodeficiency	204	F	61
10	Common variable immunodeficiency	170	M	121
11	Transient hypogammaglobulinemia of infancy	30	F	26
12	Transient hypogammaglobulinemia of infancy	25	M	21
13	Transient hypogammaglobulinemia of infancy	24	M	18
14	Transient hypogammaglobulinemia of infancy	24	M	20
15	Transient hypogammaglobulinemia of infancy	19	M	15
16	Transient hypogammaglobulinemia of infancy	18	F	12
17	Transient hypogammaglobulinemia of infancy	17	M	13

* Administration of IVIG was started in 1984; before that date, the patients received 280 g (Patient #1) and 310 g (Patient #2) of IMIG.

RESULTS

For each patient, the number of immunoglobulin administrations, the amount of immunoglobulin received (in grams), and the number of TTV PCRs performed are reported in Table 2. The total number of parenteral immunoglobulin ad-

TABLE 2. Number of immunoglobulin administrations, amount of immunoglobulins received (g), and number of TTV PCRs performed in 17 patients with congenital immunodeficiency who received monthly IVIG doses

Patient	Number of immunoglobulin administrations	Immunoglobulins received (g)	Number of TTV PCRs performed	Age of patients at TTV PCR (months)
1	302	6060	8	144, 168, 212, 237, 298, 331, 348, 357
2	283	5100	5	91, 163, 220, 275, 343
3	145	1210	7	23, 46, 88, 106, 117, 132, 141
4	42	289	4	12, 22, 29, 48
5	14	43	2	10, 16
6	74	481	4	13, 24, 36, 70
7	148	1040	7	37, 48, 61, 85, 99, 106, 138
8	123	1081	7	24, 38, 45, 68, 93, 104, 120
9	65	1500	4	145, 168, 181, 201
10	129	1502	7	50, 63, 77, 98, 123, 138, 163
11	26	98	3	6, 10, 28
12	21	74	3	7, 13, 23
13	18	77	2	9, 15
14	18	72	3	8, 20
15	14	50	2	6, 17
16	14	43	2	7, 18
17	9	53	2	5, 16

ministrations in the 17 patients was 1445 and the total amount of immunoglobulin administered was 18,773 g. The median number of TTV PCRs performed in each patient was 4 (range, 2-8).

TTV DNA was not found in any of the serum samples analyzed. None of the 15 immunoglobulin preparations analyzed was found positive for TTV DNA. All IVIG samples and pooled sera to which positive serum was added were found positive for TTV DNA.

DISCUSSION

The present results show that TTV is not transmitted through IVIG: more than 18,000 g of immunoglobulin was administered to the patients, and no case of TTV infection was recorded. These results could be due to the absence of TTV in plasma pools. However, with the same primers, the prevalence of TTV in a normal population of blood donors in Italy is 22 percent.[7] Therefore, given the high prevalence of TTV infection in blood donors and plasma pools[7,12] and the huge number of transfusions received by the patients, the absence of infection is more likely due to the removal or inactivation of TTV by the manufacturing process. This is confirmed by studies conducted by other authors. Pisani et al.,[12] with a set of primers derived from a conserved region of TTV, could detect TTV DNA in 70 percent of four plasma pools derived from blood donors from different countries, whereas IVIG was consistently negative with the same primer set. The same authors demonstrated that the large majority of FVIII concentrates and a high number of IMIG batches are TTV positive. The results obtained in the present research confirm that, unlike FVIII concentrates, IVIG preparations are free of TTV sequences.[14]

At present, most IVIG products released in the world are subjected to a virus-inactivation step using S/D treatment or pasteurization.[9,10,15-17] We cannot exclude that other methods of virus inactivation may be less effective in eliminating TTV or other blood-borne viruses. Six patients were already receiving IVIG substitute therapy before 1994, and the present results suggest that TTV was probably inactivated also by the procedures performed before that time. Moreover, as has previously been described for other nonenveloped DNA viruses,[17] TTV virus may be removed during a purification step before S/D treatment or pasteurization.

Recently, it has been suggested that the use of different primer sets or longer times of extension or annealing during PCR may increase the sensitivity of the method, but the same authors also suggested the need for development of international standards.[18]

The immunologic disorders of the patients studied, who were unable to produce immunoglobulins, precluded the evaluation of TTV antibodies. Therefore, the present data cannot exclude, in older patients, transient TTV viremia. However, this possibility seems extremely unlikely, because, as has been repeatedly shown, TTV DNA persists through a follow-up of years in infected patients.[7,19,20] In particular, in multiply transfused patients, a chronic infection may be observed in more than 80 percent of the cases,[7,20] and TTV DNA has been shown to persist in serum samples taken up to 6 years apart in individual patients.[19] Moreover, virus clearance is usually decreased in immunodeficient patients; therefore, the hypothesis of transient viremia and resolution of TTV infection is even more unlikely in these patients than in an immunocompetent host.

In conclusion, the present findings suggest that the fractionation process and stringent procedures to inactivate viruses make commercial IVIG safe and unable to transmit TTV.

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