

Hepatitis A virus antibodies in immunoglobulin preparations

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Background: Persons with primary immune deficiency receive intravenous immunoglobulin (IVIG) as antibody replacement therapy. These patients depend on the presence of protective antibody levels against circulating pathogens in IVIG.

Objectives: The incidence of hepatitis A virus (HAV) infections has been decreasing globally.

We investigated whether this decrease in HAV incidence is reflected in human plasma pools and evaluated whether HAV antibody titers in IVIG preparations are still adequate for antibody replacement.

Methods: By using ELISA, the HAV antibody titer of 3,953 plasma pools sourced from March 2003 through September 2008 in the European Union (EU) or United States (US) and of 169 IVIG lots manufactured from 2005 through 2007 was determined. The functionality of the HAV antibodies contained in IVIG was assessed by using a microneutralization assay.

Results: The results confirm a decrease in HAV antibody titers in EU (−28%) and US (−41%) plasma. Furthermore, the mean HAV antibody content in EU (1.70 ± 0.12 IU/mL) and US (0.82 ± 0.09 IU/mL [mean $\pm$ SEM]) plasma was significantly different ($P = .0001$). A significant difference ($P < .0001$) was also evident in the IVIG preparations KIOVIG (22.91 ± 0.68 IU/mL) and Gammagard Liquid (14.60 ± 0.48 IU/mL), respectively, made from EU or US plasma. In accordance with the ELISA results, there was a significant difference ($P < .0001$) in HAV neutralization titer 50% (NT₅₀) values between IVIG produced from EU-sourced ($2,477 \pm 265$ NT₅₀ [1:X]) or US-sourced (844 ± 82 NT₅₀ [1:X]) plasma.

Conclusion: Although HAV antibody seroprevalence continues to decrease in Europe and the US, HAV antibody titers in IVIG lots appear to remain adequate for antibody replacement therapy. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Intravenous immunoglobulin, IGIV, immune globulin, antibody functionality, replacement therapy, hepatitis A incidence, seroprevalence, virus neutralization, human plasma pool

Intravenous immunoglobulin (IVIG) is a therapeutic preparation containing the pooled antibodies (mainly IgG) from blood

Abbreviations used

EU: European Union
HAV: Hepatitis A virus
IVIG: Intravenous immunoglobulin
NT₅₀: Neutralization titer 50%
PID: Primary immunodeficiency
US: United States

and plasma donors. One of the main areas of IVIG application is as antibody replacement therapy in persons with primary immunodeficiencies (PIDs).¹ These patients critically depend on the presence of a variety of antibody specificities in IVIG to ensure continued protection against any viral or microbial pathogens they might encounter. Legal product specifications include minimum antibody level requirements for diphtheria, measles, and a poliomyelitis virus in the United States (US)² and for hepatitis B virus antibody levels in Europe^{3,4} to guarantee a certain level of antibody specificity in IVIG. However, no requirements for hepatitis A virus (HAV) antibody levels are specified for either area. Because IVIG is manufactured from the variable resource human plasma, lot-to-lot variation in antibody levels of IVIG products are inevitable and have been reported.⁵

Human plasma pools and consequently the immune globulin preparations fractionated thereof represent the immune status of thousands of healthy plasma donors. They are therefore valuable seroepidemiologic tools, as recently shown through a demonstration of the gradual US seroconversion after introduction of the West Nile virus in 1999⁶ or through the determination of population segments with increased human parvovirus B19 antibody levels.⁷

Infection with HAV has variable outcomes, ranging from asymptomatic infection to fulminant hepatitis. The disease occurs worldwide and remains of economic importance; a recent analysis estimated costs of US\$133.5 million during the lifetime of a single age cohort of children born in 2005 in the US.⁸ For the past 50 years, the incidence of hepatitis A infection has been in decline throughout the world,⁹⁻¹¹ and factors contributing to this decrease are (1) increase in socioeconomic status, (2) improvement of water hygiene, and (3) availability of an effective HAV vaccine since 1995.^{9,12} The available data on HAV antibody seroprevalence are outdated, originating mainly from times before the introduction of HAV vaccines. The most current available information on HAV antibody seroprevalence in the US, the single most important source of human plasma for fractionation, was compared with the HAV antibody occurrence in some European countries (Table I).^{9,13-15} As evident from these previously reported results, the seroprevalence of HAV seems to be lower in the US population.¹³⁻¹⁶

Little is known about the effect the continuous decrease in hepatitis A incidence and the introduction of HAV vaccines had

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TABLE I. HAV antibody seroprevalence (percentage of population) in the US, Austria, Germany, and the Czech Republic

Country	Period surveyed	Mean	Reference
US	1988-1994	31.3	Wasley et al, 2005 ⁹
Austria	1985-1992	47.0*	Prodinger et al, 1994 ¹³
Germany	1998	46.5	Thierfelder et al, 2001 ¹⁴
Czech Republic	2003-2004	61.6	Chlibek et al, 2006 ¹⁵

*The mean was calculated from data provided by the authors.

on the HAV antibody abundance and reactivity in IVIG preparations or whether the present HAV antibody levels in IVIG still provide protection for persons with PIDs. Because HAV vaccination is inefficient for the prevention of HAV infection in persons with PIDs, these patients rely on the presence of protective HAV antibody levels in IVIG. We therefore aimed to evaluate differences in the quantity and functionality of HAV antibody content of European Union (EU)- and US-sourced human plasma pools and IVIG preparations made thereof.

METHODS

Virus and cells

HAV strain HM175/24a was obtained from Dr S. Emerson (National Institute of Allergy and Infectious Diseases, National Institutes of Health), propagated on BSC-1 cells (European Collection of Cell Cultures 85011422) and titrated on an in-house strain of FRhK4 cells.

Plasma pools, IVIG preparations, and determination of HAV antibody titers by means of ELISA

The HAV antibody titers of all EU-derived (sourced mainly from Austria, Germany, and the Czech Republic) and US-derived plasma pools used in manufacture at Baxter AG (Vienna, Austria) between March 2003 and September 2008 were determined by means of ELISA ($n = 3,953$) by using the Enzygnost anti-HAV kit (Dade Behring, Marburg, Germany) and following the manufacturer's instructions. In addition, the HAV antibody titers of 24 EU plasma-derived IVIG lots (KIOVIG; Baxter AG, Vienna, Austria) and 145 US plasma-derived IVIG lots (Gammagard Liquid; Baxter Healthcare Corp, Westlake Village, Calif) manufactured in 2005 to 2007 were determined by means of ELISA. KIOVIG and Gammagard Liquid are 10% liquid IVIG preparations primarily for the European and American markets, respectively, and manufactured by means of the same process.¹⁷ Accordingly, the respective plasma pools that were sourced in 2005 to 2007 were identified ($n = 1,604$).

Microneutralization assay and virus titration

Microneutralization assays for IVIG were done in at least 2 independent replicates and read by 2 different operators. Microneutralization was done essentially as in the study by Ehrlich et al,¹⁸ with the following modifications: IVIG samples were initially diluted 1:10, 2×10^3 mean tissue culture infectious doses 50% per milliliter of HAV were added to each sample, and incubation on FRhK4 cells was done for 21 days, with partial media changes at days 7 and 14. After 3 weeks, each well was assessed for virus-induced cytopathic effects, and the microneutralization titer 50% (NT₅₀) was calculated. Results were rounded to the nearest integer.

Statistical analysis

Graphs and statistical analysis were done with GraphPad Prism version 5.0 software (San Diego, Calif). Differences in plasma pool and IVIG antibody content were analyzed by using the Student *t* test (unpaired and 2-tailed), and *P* values, difference of the means as well as the 95% CI, are reported; comparison of variance was done with the *F* test. Where appropriate, the difference in plasma pool antibody titers was also analyzed by using a 1-way ANOVA,

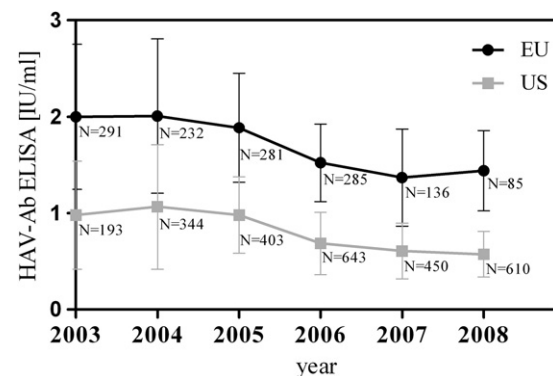


FIG 1. ELISA-determined HAV antibody (Ab) content of EU- and US-derived plasma pools used in manufacture at Baxter AG (Vienna, Austria) between March 2003 and September 2008. *N*, Number of plasma pools. Error bars indicate SDs.

where the variance within groups was compared with the Bartlett test. Correlation was analyzed assuming Gaussian distribution (Pearson), and the 2-tailed *P* value was stated.

RESULTS

HAV antibody titers in EU- and US-derived plasma pools

There was a significant difference ($P = .0001$; 0.89; 95% CI, 0.56-1.22) of average HAV antibody titers during the period from 2003 to 2008 in EU- versus US-derived plasma pools (Fig 1). The average HAV antibody content of EU plasma pools sourced from 2003 to 2008 was 1.70 ± 0.12 IU/mL (mean $\pm$ SEM; $n = 1,310$), whereas US plasma pools contained 0.82 ± 0.09 IU/mL ($n = 2,643$). The significant difference of HAV antibodies in EU- and US-sourced plasma pools was also evident when the average HAV antibody content of 2003, 2004, 2005, 2006, 2007, and 2008 was compared separately; each year, the HAV antibody titers of US-derived plasma pools were significantly ($P < .001$) lower.

Determination of the HAV antibody content in plasma pools from 2003 versus 2008 showed a significant decrease (EU: $P = .0001$; 0.56; 95% CI, 0.39-0.72; US: $P = .0001$; 0.41; 95% CI, 0.35-0.46) in HAV antibody titer in EU plasma pools of -28% (2003: 2.00 ± 0.04 IU/mL; 2008: 1.44 ± 0.05 IU/mL) and of -41% in US plasma pools (2003: 0.98 ± 0.04 IU/mL; 2008: 0.57 ± 0.01 IU/mL; Fig 1). During the 6-year time period surveyed in this study, the HAV antibody titer in US plasma pools was consistently only around 50% of that detected in EU plasma pools (Fig 1).

When the average HAV antibody content of plasma pools sourced entirely in Germany ($n = 15$, 1.23 ± 0.09 IU/mL), Austria ($n = 17$, 1.34 ± 0.09 IU/mL), or the Czech Republic ($n = 8$, 1.15 ± 0.23 IU/mL) in the period of 2007 and 2008 was compared, no significant differences were found between titers of these EU countries (Germany vs Austria, $P = .4$; Germany vs Czech Republic, $P = .7$; Austria vs Czech Republic, $P = .4$).

HAV antibody titers in EU- and US-derived IVIG preparations

When only the reduced sample size selection of plasma pools sourced from 2005 to 2007 was analyzed, the mean HAV antibody

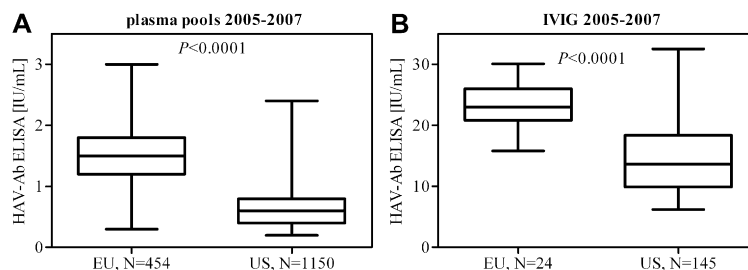


FIG 2. Comparison of 2005 to 2007 HAV antibody (Ab) content in EU- or US-derived plasma pools for manufacture (A) and EU- or US-derived IVIG final containers (B). Error bars indicate minimum and maximum ELISA titers, and the middle line indicates the mean.

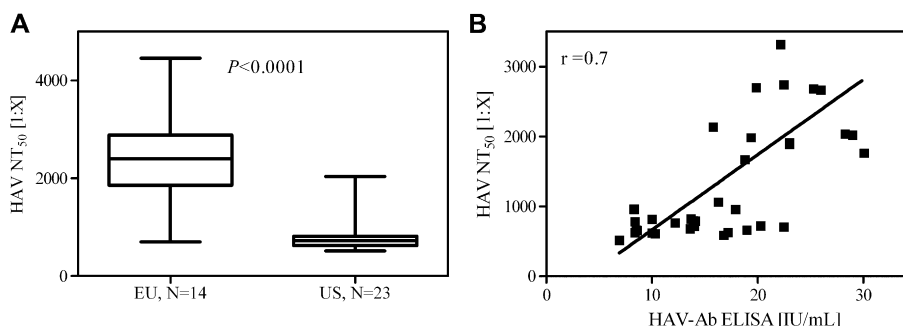


FIG 3. Comparison of the NT₅₀ value of EU- and US-derived IVIG final containers (A) and linear regression analysis of IVIG HAV antibody (Ab) ELISA titers versus neutralization titers (B). Error bars indicate minimum and maximum NT₅₀ values, and the middle line indicates the mean.

titer between EU- versus US-derived plasma pools was also significantly different ($P < .0001$; 0.82; 95% CI, 0.79-0.86; Fig 2, A). The average HAV antibody content in these EU- or US-derived plasma pools was 1.49 ± 0.02 IU/mL ($n = 454$) and 0.67 ± 0.01 IU/mL ($n = 1,150$), respectively (Fig 2, A). This significant difference in mean plasma HAV antibody content was also reflected in IVIG final containers manufactured from 2005 to 2007 from EU (22.91 ± 0.68 IU/mL, $n = 24$) or US (14.60 ± 0.48 IU/mL, $n = 145$) plasma ($P < .0001$; 8.31; 95% CI, 5.92-10.70; Fig 2, B).

HAV microneutralization

For the significantly more laborious and biohazardous HAV microneutralization, a subset of the IVIG lots tested by means of ELISA was chosen (14 of the 24 EU-derived and 23 of the 145 US-derived IVIG lots). The subset chosen for microneutralization was statistically equivalent to the total number of IVIG lots tested by means of ELISA. Specifically, the average HAV antibody content in the reduced sample size did not differ significantly from that in the large sample size (EU derived: 22.77 ± 0.97 IU/mL, $n = 14$, $P = .9$; US derived: 14.16 ± 1.25 IU/mL, $n = 23$, $P = .7$), whereas the difference in HAV antibody titers between the small sample size EU- and US-derived IVIG lots remained significant ($P < .0001$).

The variation in HAV NT₅₀ values was different between EU- and US-derived IVIG lots ($P = .0002$, F test). The mean HAV NT₅₀ value differed significantly between EU-derived ($2,477 \pm 265$ [mean $\pm$ SEM], $n = 14$) and US-derived (844 ± 82 , $n = 23$) IVIG lots ($P < .0001$; 1633; 95% CI, 1165-2101; Fig 3, A). To enhance the robustness of statistical evaluation, the values

were also analyzed after log-transformation, and the difference in HAV NT₅₀ values remained significant ($P < .0001$; log 0.46; 95% CI, log 0.34-0.58).

Already indicated by the difference in HAV NT₅₀ values between EU- and US-derived IVIG lots, significant correlation ($r = .7$, $P < .0001$) of ELISA-determined HAV antibody titers and HAV NT₅₀ values was seen (Fig 3, B).

DISCUSSION

For the past 50 years, a worldwide decrease in hepatitis A incidence has been reported.⁹⁻¹¹ Our results indicate that this decrease in disease incidence is continuing because we detected a further decrease of around one third in human plasma HAV antibody content within the 6-year time period surveyed (Fig 1).

Our results further indicate a significant difference in HAV antibody prevalence in the plasma donor populations of the US and the EU. Plasma donors are selected according to strict eligibility criteria, which are practically identical in the US and the EU (ie, a medical history of viral hepatitis is an exclusion criterion).¹⁹ The observed intercontinental differences in IVIG HAV antibody titers can thus not be explained by different donor selection criteria. However, one factor contributing to this difference might be the differential HAV vaccination strategies in the US and the EU. After infection with HAV, the serum HAV antibody titer is 3- to 30-fold higher (>11.4 IU/mL) than after HAV vaccination (0.4-4.0 IU/mL).²⁰⁻²³ Especially in the US, where HAV vaccination of all children has been added to the schedule of routine childhood vaccines,⁸ a further decrease in HAV antibody seroprevalence can be expected because fewer natural

infections occur. On the contrary, routine HAV vaccination has not been added to the immunization schedule of Austria, Germany, or the Czech Republic; in these countries HAV vaccination is only recommended for persons at specific risk of infection with HAV.²⁴⁻²⁷ Because the European recommendations will invariably result in lower HAV vaccination coverage, the future decrease in EU plasma HAV antibody titer should be less than in the US because HAV infections can be expected to occur more frequently.

Our results show that the HAV antibody titer of EU-sourced plasma was approximately double that of US-sourced plasma (Fig 1), therefore indicating a more frequently occurring HAV infection in the EU countries from which the plasma was sourced (mainly Austria, Germany, and Czech Republic). Of these countries, Austria and Germany are listed as countries of low HAV antibody prevalence, whereas the Czech Republic remains in the intermediate prevalence category.¹² However, this categorization was not confirmed by our results. Comparing the average HAV antibody content in plasma pools entirely comprised of Czech donations to pools sourced entirely in Austria or Germany, no significant difference in antibody titers was observed. This result indicates that the level of HAV infection among plasma donors, as opposed to the general population, is approximately equal in these 3 EU countries. It will be interesting to follow the future development of HAV antibody prevalence in the different European plasma-sourcing countries and intriguing to see whether large-scale hepatitis A outbreaks, as recently reported in Prague (Czech Republic),²⁸ have a detectable effect on HAV antibody titers in human plasma pools. Equally, it will be of interest to see whether HAV antibody titers of plasma pools further decrease in the US when the first birth cohorts who have generally received HAV vaccination will commence to donate blood or plasma. A similar association of vaccine-induced immunity and concomitant decrease in specific antibody titers has already been associated with measles vaccination and the decrease of measles antibodies in US IVIG.²⁹

Because the IVIG preparations made of EU and US plasma are both manufactured using the same process,¹⁷ it was not surprising that the difference in HAV antibody content in EU- versus US-sourced human plasma was reflected in the IVIG preparations made thereof (Fig 2, B). A similar difference in HAV antibody content has previously been noted during an evaluation of antibody quantities in United Kingdom- and US-sourced immune globulins.³⁰

Although the HAV antibody content was significantly lower in US-sourced IVIG, the average US HAV antibody titer of 14.60 IU/mL appears to be adequate for clinical applications, considering that levels of 10 mIU/mL or greater HAV antibody in immunoglobulin preparations are thought to be protective.^{20,31} In fact, these numbers indicate that the average US IVIG HAV antibody titers are more than 1000-fold greater than the minimum IVIG level required to prevent infection by HAV. Further evidence for sufficient HAV antibody levels in US IVIG comes from the relation of administered HAV antibody quantity to dilution of the antibodies in the body. A person with PID and body weight of 80 kg receives 1,488 IU of HAV antibody every 3 to 4 weeks, assuming that the smallest recommended dosage of 0.3 g/kg Gammagard Liquid containing the lowest HAV antibody titer measured in this survey (6.2 IU/mL) is administered. Because it was previously shown that 700 IU of HAV antibody administered in immunoglobulin was sufficient to maintain a serum HAV antibody titer of greater than 22 mIU/mL for more than 2 months in healthy

volunteers,²⁰ a further 50% decrease in US IVIG HAV antibody titer would still result in an immunoglobulin product with protective HAV antibody levels. Because HAV antibody titers are expected to continue to decrease in both EU- and US-sourced plasma, the clinical effect on PID treatment with lower HAV antibody titer IVIG should be closely monitored in the future.

The significant difference in HAV antibody content between EU- and US-sourced IVIG was reflected in a significantly different HAV-neutralization capacity (Fig 3, A), where the higher HAV antibody titer in EU-sourced IVIG does have greater neutralization capacity. These results are in accordance with data obtained in previous studies, in which the neutralization activity of HAV antibodies raised in persons receiving HAV vaccines was investigated and good correlation between ELISA titers and neutralization activity within vaccinated groups was reported.^{22,32}

In conclusion, we showed the suitability of using pooled human plasma and immune globulin preparations to investigate the serostatus of different nationalities and population segments, exemplified here with an evaluation of HAV antibody prevalence in the US and countries of the EU. Our results add further evidence to a decrease of HAV incidence in the US and the EU. Most importantly, though, we showed that although HAV incidence continues to decrease, HAV antibody titers in IVIG remain at protective levels and therefore appear adequate for antibody replacement therapy in patients with PIDs.

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Key messages

- IVIG made from European plasma has significantly higher HAV antibody titers and greater HAV-neutralization capacity than IVIG made from US plasma.
- Although HAV antibody seroprevalence continues to decrease in Europe and the US, IVIG HAV antibody titers appear to remain adequate for antibody replacement therapy.

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