Vaccine 33 (2015) 3600–3604

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Persistence of antibodies six years after booster vaccination with inactivated vaccine against Japanese encephalitis

Maria Paulke-Korinek[a,b], Herwig Kollaritsch[a,*], Michael Kundi[c], Ines Zwazl[a], Claudia Seidl-Friedrich[a], Tomas Jelinek[d]

[a] Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Kinderspitalgasse 15, A-1090, Vienna, Austria
[b] Federal Ministry of Health, Austria
[c] Institute of Environmental Health, Center for Public Health, Medical University of Vienna, Kinderspitalgasse 15, A-1090, Vienna, Austria
[d] Berlin Center for Travel and Tropical Medicine, Berlin, Germany

ARTICLE INFO

Article history:
Received 19 February 2015
Received in revised form 10 May 2015
Accepted 17 May 2015
Available online 30 May 2015

Keywords:
Japanese encephalitis vaccine
Long-term protection
Low responder

ABSTRACT

Background: Japanese Encephalitis (JE) virus occurs in wide regions of Asia with over 3 billion people living in areas at risk for JE. An estimated 68,000 clinical cases of JE occur every year, and vaccination is the most effective prophylactic measure. One internationally licensed vaccine containing the inactivated JE virus strain SA_{14}-14-2 is Ixiaro® (Valneva, Austria). According to recommendations, basic immunization consists of vaccinations on day 0, day 28, and a booster dose 12–24 months later. Protection in terms of neutralizing antibody titers has been assessed up to 12 months after the third dose of the vaccine. The current investigation was designed to evaluate antibody decline over time and to predict long-term duration of seroprotection after a booster dose.

Method: In a preceding trial, volunteers received basic immunization (day 0, day 28) and one booster dose against JE 15 months later. A follow up blood draw 6 years following their booster dose was carried out in 67 subjects. For antibody testing, a 50% plaque reduction neutralization test ($PRNT_{50}$-test) was used. $PRNT_{50}$ values of 10 and above are surrogate levels of protection according to WHO standards.

Result: Seventy-six months following the booster dose, 96% of the tested subjects had $PRNT_{50}$ titers of 10 or higher. Geometric mean titer (GMT) was 148 (95% CI confidence interval: 107–207). Antibody titers were lower in volunteers 50 years of age and older. Vaccination history against other flaviviruses (yellow fever or tick borne encephalitis) did not significantly influence $PRNT_{50}$ titers. A two-step log-linear decline model predicted protection against JE of approximately 14 years after the booster dose.

Conclusion: Six years after a booster dose against JE, long-term protection could be demonstrated. According to our results, further booster doses should be scheduled 10 years following the first booster dose.

1. Introduction

Japanese Encephalitis, JE, is caused by the Japanese Encephalitis Virus, JEV. The disease is endemic in many countries in Asia harboring a population of more than 3 billion [1,2]. This also is the region with highest annual growth in international tourism; in 2013, 248 million tourists were registered in this region [3].

The pathogen causes at minimum 68,000 annual episodes of JE and is the most important virus responsible for encephalitis in Asia [4,5]. Furthermore, it is a relevant disease in travel medicine: in the past, JEV has caused several cases of JE in travelers [6–8]. There is no specific antiviral treatment for JE, so prevention is even more important. Aside from the use of repellents, the only way to prevent JE is prophylactic vaccination, which is recommended for travelers going to endemic regions for longer periods of time (4 weeks or more) and for travelers performing extensive outdoor activities [2].

There are two internationally licensed vaccines for travelers to Asia to prevent JE called Ixiaro® (approved in Europe, some countries in Asia and USA, brand name Jespect® in Australia; Valneva Austria GmbH, Vienna, Austria), and Imojev® (live-recombinant (chimeric) vaccine, Sanofi-Pasteur, Lyon, France, approved in Australia and some Asian countries). Ixiaro® is a vero-cell derived

* Correspondence to: Institute of Specific Prophylaxis and Tropical Medicine, Center for Physiology, Pathophysiology and Immunology, Medical University of Vienna, Kinderspitalgasse 15, A-1090 Vienna, Austria. Tel.: +43 1 40160 38200; fax: +43 1 40160 938293.
E-mail address: herwig.kollaritsch@meduniwien.ac.at (H. Kollaritsch).

http://dx.doi.org/10.1016/j.vaccine.2015.05.037
0264-410X/

whole-virus alum adjuvanted vaccine containing the inactivated strain SA_{14}-14-2 adsorbed to Alum. Its high immunogenicity and safety has been demonstrated in clinical trials and post-marketing use [9–11]. Recommended basic immunization schedule consists of three vaccinations: day 0 and day 28, followed by a booster vaccination 12–24 months later [12]. To date, there is no recommendation for consecutive booster doses. Mathematical models predicted protection after the booster for at least 4 years in 95% of the subjects and protection for 8 years in 50% of the tested subjects [13]. However, until today there are no clinical data verifying these calculations, and immunity after the booster dose in adults has been investigated only up to 12 months after booster (dose 3) [14]. Information on long term immunity after JE vaccination is needed to provide recommendations for scheduling further booster doses.

The present study was designed to assess the antibody decline approximately 6 years after a booster dose against JE with inactivated vaccine and to predict long-term duration of seroprotection in a population vaccinated in a preceding vaccine trial against JE. Furthermore, influence of age and gender on antibody titers was assessed and it was tested whether history of vaccination against flavivirus infection such as tick borne encephalitis (TBE) or yellow fever (YF) had an impact on antibody titers.

2. Methods

2.1. Study design

In this study, 155 subjects from a preceding trial were invited for a blood draw 76 months after their booster dose against JE. In the preceding trial, subjects received basic immunization and one booster dose approximately 15 months later. Study participants were assigned to different age groups (<50; 50 years and older) according to their age at the time of the booster vaccination. Grouping for vaccination history regarding YF and TBE depended on the vaccination history at any time before the blood draw at month 76 (in some cases before, in some cases after vaccination against JE). History of travel to JE endemic countries since the booster dose was collected.

Subjects were invited at one study site in Austria and one study site in Germany. The study was conducted according to GCP, a positive opinion by the responsible ethics committees was available and all subjects provided informed consent.

2.2. Laboratory procedures

After sampling, sera were stored below −20 °C until analysis. Sera were analyzed using a 50% plaque reduction neutralization test ($PRNT_{50}$-test) at Valneva Austria GmbH as described previously [13]. $PRNT_{50}$ values of 10 were regarded the surrogate level of protection according to World Health Organization, WHO.

3. Statistical analysis

Based on the variability of $PRNT_{50}$ titers determined at previous time points 35 patients from each of the two centers are sufficient to detect the predicted titer decrease with 90% power at the 5% significance level. As only 67 subjects could be included the expected power is marginally reduced to 88%.

Data were entered into a Microsoft Excel 2010 database and evaluated by Graph Pad Prism 4.0 and SPSS 22.0. For calculations of geometric mean titer, GMTs, titers below the cut off $PRNT_{50}$ of 10 were set at 5. The seroprotection rate, SPR, was the rate of subjects with JEV neutralizing antibody titers ≥1:10 in a $PRNT_{50}$. For illustrative purposes, we calculated the geometric mean titer stratified by tick-borne encephalitis vaccination history, yellow fever vaccination history, gender and age groups. Log titers were tested by a general linear model with gender, age group and history of vaccination against other flaviviruses as factors. Antibody decline was analyzed using previously measured $PRNT_{50}$ titers during follow up starting with the titer obtained approx. 4 weeks after booster. Based on the previous analysis [14], a breakpoint at 6 months was applied and the dependency of log titers on months of follow up determined by a General Estimation Equation (GEE) model with an unstructured correlation matrix and gender, age, and history of vaccination against other flaviviruses as covariates.

Individual titer results determined in preceding studies were obtained from the manufacturer and the mean duration of protection after a booster dose of JE vaccine was calculated using the regression coefficients from the GEE model and the titer 4 weeks after booster as described previously [13].

Table 1
Demographic and baseline characteristics of subjects returning for a blood draw 76 months after booster vaccination.

	$N = 67$, n	%
Female	39	58
Male	28	42
Age <50 years	61	91
Age 50 years and above	6	9
TBE vaccination	31	46
No TBE vaccination	36	54
YF vaccination	5	8
No YF vaccination	61*	92

* In one subject, YF vaccination history was unknown.

4. Results

Blood samples were drawn from 67 study participants (34% of the subjects originally vaccinated in a preceding trial; 43% of the invited subjects) with a mean age of 38.8 (±11.0 SD) years at the time of the blood draw. 58% of the subjects were females. Demographic data are summarized in Table 1. Their last booster dose was on average 75.5 (SD ±1.2; min 72.8 months, max 77.4 months) months earlier. Seventeen subjects reported history of travel to JE endemic countries.

4.1. Geometric mean titers and estimated duration of protection

The $PRNT_{50}$ GMT was 148 (95% CI: 107–207), and 96% of the tested subjects had protective antibody titers (i.e., $PRNT_{50}$ titers ≥10). GMTs and SPRs since primary immunization are shown in Table 2 together with titers obtained at previous time points. On average, GMTs were higher in females (GMT 168; 95% CI 106–264) than in males (GMT 125; 95% CI 76–207) (not statistically significant, $p = 0.372$, Fig. 1). GMTs in subjects 50 years of age and older at time of booster vaccination ($n = 6$) were lower (GMT 52; 95% CI 10–269) compared to antibody titers in subjects below 50 years (GMT 165; 95% CI 118–230), this trend was statistically significant ($p = 0.048$, Fig. 1).

According to mathematical modeling using a log linear function with a structural break at 6 months, average protection is estimated to last for a total of 14 years after the booster dose (range 2–25 years) (Fig. 2), which is twice as long as originally predicted after analysis of 12-months antibody titers. Table 3 shows the estimated duration of protection for different titer levels 4 weeks after the booster dose.

4.2. Vaccination against tick-borne encephalitis and yellow fever

Thirty-one subjects were previously vaccinated against TBE and 36 subjects had no TBE vaccination history. $PRNT_{50}$ GMT in subjects vaccinated against TBE were lower with values of 127 (95% CI

Table 2
GMT for JE neutralizing antibodies and Seroconversion rates (SCR) after primary series and booster, (Confidence interval CI).

Time point	GMT	95% CI	SCR	95% CI
Post primary series				
Day 56	183	[136; 246]	97% (65/67)	90%; 100%
Month 15	20	[15; 27]	96% (64/67)	87%;99%
Post booster				
Day 28	927	[651; 1321]	100% (67/67)	95%;100%
Month 6	514	[350; 754]	99% (66/67)	92%; 100%
Month 12	353	[240; 519]	99% (66/67)	92%; 100%
Month 76	148	[107; 207]	96% (64/67)	87%; 99%

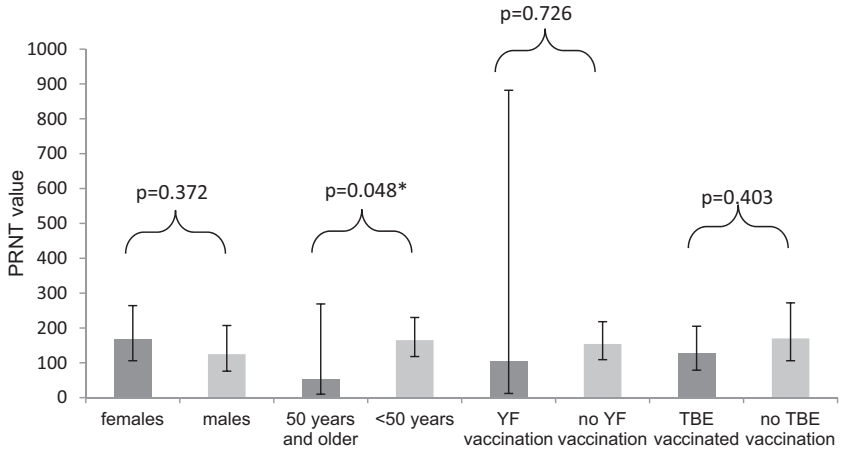

Fig. 1. $PRNT_{50}$ GMTs in males and females, subjects below 50 years and 50 years of age and older, subjects with history of yellow fever (YF) vaccination and without YF vaccination history as well as subjects with tick borne encephalitis (TBE) vaccination history and without TBE vaccination history. *P*-values <0.05 are considered statistically significant (*).

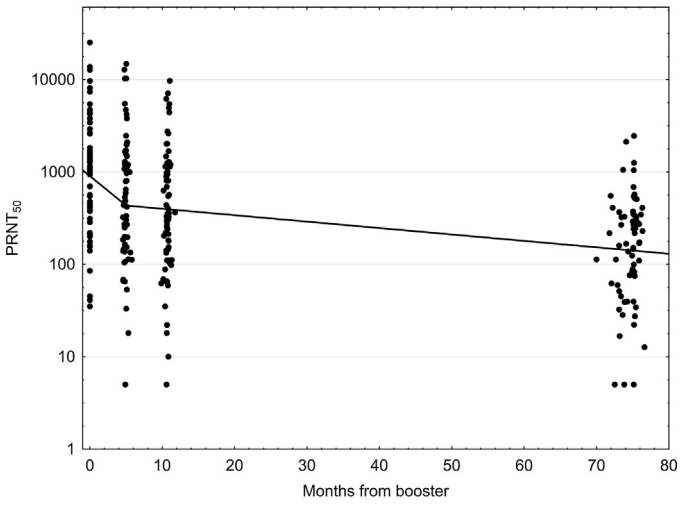

Fig. 2. Titers post booster in 67 volunteers followed for 76 months. Estimated titer decline using a log linear function with a structural break at 6 months is shown.

Table 3
Examples of post-booster titers and the associated predicted duration of protection and its 95% confidence interval.

Titer after the booster	Estimated duration of protection in years	95% CI
100	7.4	[6.7;8.1]
500	12.5	[11.3;13.8]
900	14.4	[13.0;15.8]
1000	14.8	[13.3;16.2]
1500	16.1	[14.5;17.6]

79–205; mean age 33.6 ± 11.5 years) compared to subjects without history of TBE vaccination (GMT 170; 95% CI 106–272; mean age 31.7 ± 10.6 years), but the difference was not statistically significant ($p = 0.403$, Fig. 1).

YF vaccination history was present in five subjects, 61 subjects were not vaccinated against YF and in one person YF vaccination history was unknown. GMTs in persons with YF vaccination history were 105 (95% CI 12–882) compared to persons without YF vaccination (GMT 154; 95% CI 109–218); the difference was not statistically significant ($p = 0.726$, Fig. 1).

No statistically significant difference in antibody titers in subjects with or without prior exposure to TBE or YF vaccination was observed (Table 4).

4.3. Subjects with $PRNT_{50}$ titers below 10 "low responders"

In three subjects, $PRNT_{50}$ antibody titers were below the cut off level of 10 at month 76. None of them was vaccinated against YF or traveled to JE-endemic regions since the booster dose. Two of them

Table 4
GMTs for JE neutralizing antibodies and estimated duration of protection (months) and 95% confidence intervals in participants with and without history of TBE or YF vaccination. p-values from analysis of variance with linear contrasts.

	History of TBE or YF vaccination		
	W/o	With	
Post-Booster	*n* = 35	*n* = 32	*p*-value
Day 28	1204 (756; 1918)	697 (403; 1205)	0.117
Month 6	587 (356; 970)	444 (241; 818)	0.421
Month 12	471 (284; 782)	257 (142; 466)	0.083
Month 76	164 (101; 265)	133 (83; 214)	0.551
Duration of protection	175 (156; 195)	152 (130; 175)	0.124

were aged 35 years (one vaccinated against TBE, last vaccination in 2010); the third person was 51 years old at the time of the booster dose. Neither of them had a relevant medical history. None of these subjects had detectable titers before the booster but all developed protective titers 4 weeks after the booster. One of these subjects already was seronegative 6 months after the booster. Within error of measurement, their antibody kinetics followed a one component decline, i.e., they lacked the second component that results in a slowing down of antibody loss.

5. Discussion

This is the first clinical study presenting data on the long term immunity up to 76 months after the booster dose (delivered 15 months following primary vaccination) with inactivated JE vaccine SA_{14}-14-2. Six years after the booster dose, $PRNT_{50}$ GMTs still were 148 and 96% of the tested subjects had $PRNT_{50}$ antibody titers above 10, the surrogate level of protection.

Our study population represents about one-third of the original population included in the preceding trial, but seems to reflect the original population very well. GMTs of our study cohort at earlier time points are very comparable to GMTs published for the original larger cohort [13]. Mathematical modeling from titers up to 12 months after booster predicted a protection rate of 95% 4 years following the booster dose and a protection of 50% of the subjects after 8 years [13]. However, these estimates were conservative since for the slow component of antibody decline only the time point 12 months after booster was available. Therefore, the observational period was extended to get a more precise estimate of antibody decline and to determine the optimal time point for a further booster vaccination. From follow up data about 6 years after the first booster dose, we predict an average period of protection of 14 years. Correlates of protection (i.e., $PRNT_{50} \geq 10$) were detected in 96% of the tested subjects 6 years after the booster dose and it is estimated that 75% of the subjects will be protected for 10 years or longer.

As a sign of immunosenescence, immune response is impaired in the elderly after vaccination, such as vaccinations against TBE, tetanus or JE [15–18]. This goes in line with our findings showing that antibody titers were lower in subjects 50 years of age and older (at time of basic immunization, one subject was 49 years old, the others were aged 50 years and older at priming). Since only six subjects in our study were in this older age group and considering the result from other investigations [17,18], efforts should be raised to get more insight into the influence of ageing on the immune response after JE vaccination.

Belonging to the same family of viruses, it has been discussed whether there may be any interference or cross-reactivity of antibodies against JE with those from other flaviviruses as these viruses have highly conserved envelope protein amino acid sequences [19–22]. This was addressed in an investigation also using the SA_{14}-14-2 vaccine in 420 subjects where JE antibody titers were significantly higher during basic immunization course after the first JE vaccination but not after the second in persons with anti-TBE antibodies compared to subjects without TBE vaccination [23]. TBE antibody titers, however, were not influenced by history of YF or JE vaccination in another study with approx 180 vaccinees [24]. Whether or not previous TBE or YF vaccination interfered with JE antibody response or antibody decline was also addressed in this study. There was no statistically significant difference in antibody titers in subjects with or without prior exposure to TBE or YF vaccination and also antibody decline was not affected (Table 4).

History on travel to JE endemic regions was collected and indeed, there were 17 subjects who visited endemic areas and this had the theoretic potential of a natural booster. However, in all of these subjects antibody titers followed the curve as it was expected i.e., increase after booster vaccination and afterwards decline of antibody titers. If any of these persons had a natural booster dose, this could be identified by increasing antibody titers which did not occur in any of these subjects.

There were three subjects with antibody titers below the protective threshold at months 76 after the booster dose. One of these subjects developed protective antibody titers after the basic immunization course. Each of them had protective titers 4 weeks after the booster dose but titers were again low (although above the limit of protection), and declined faster in those three persons than in the other study participants. This suggests that there is a very small proportion of primary "low-responders", in our case 4% of the tested subjects. This is a phenomenon that has also been described after other vaccinations such as TBE vaccination or hepatitis A and hepatitis B vaccination [24–28]. Antibody kinetics in these three individuals followed a one-component decline. This suggests an imbalance between memory B cells and long-lived antibody secreting plasma cells.

6. Conclusion

Long-term protection after a booster dose against JE up to 6 years following basic immunization and one booster dose with the inactivated JE vaccine containing the strain SA_{14}-14-2 could be shown. Mathematical models predict that protection will, on average, last for 14 years. Our results suggest that further booster vaccinations should be scheduled approximately 10 years after the first booster dose. This recommendation is based on an estimated rate of 75% with protective titers after 10 years and the assumption of a fast anamnestic response. Five years after the second booster, the protection rate was predicted to be 95%.

Acknowledgments

Parts of the manuscript are presented at the 14th Conference of the International Society of Travel Medicine, Quebec in May 2015.

Conflict of interest

The study was supported by Valneva, Austria GmbH with sponsoring for carrying out the study and conduction $PRNT_{50}$ tests.

MPK accepted fees for speaking, serving on advisory boards and received funding to attend conferences from Baxter, Pfizer, Novartis and GlaxoSmithKline. HK accepted educational grant fees and payment for lectures, and as an independent safety monitor in clinical studies, and reimbursement for attending meetings from GlaxoSmithKline and Pfizer. TJ accepted educational grant fees, payment for lectures, and reimbursement for attending meetings from GlaxoSmithKline, Novartis Vaccines, Baxter, Valneva and Pfizer.

References

[1] Halstead SB, Javobson J, Dubischar-Kastner K. Japanese encephalitis. In: Plotkin SA, Orenstein WA, Offit PA, editors. Vaccines. 6th ed. Saunders: Elsevier; 2013. p. 312–51.
[2] World Health Organization. International travel and health. Japanese encephalitis, ⟨http://www.Who.Int/ith/en⟩; [accessed 23.01.15].
[3] World tourism organization UNWTO. Annual report 2013. ⟨http://www2.unwto.org/publication/unwto-annual-report-2013⟩; [accessed 21.01.15].
[4] World Health Organization.Japanese encephalitis vaccines: WHO position paper. WER 2006;81, 325-40 [accessed 13.02.15]. http://www.who.int/immunization/topics/japanese_encephalitis/en/.
[5] Campbell GL, Hills SL, Fischer M, Jacobson JA, Hoke CH, Hombach JM, et al. Estimated global incidence of Japanese encephalitis: a systematic review. Bull World Health Organ 2011;89(10) [766-74E].
[6] Hills SL, Griggs AC, Fischer M. Japanese encephalitis in travelers from non-endemic countries, 1973–2008. Am J Trop Med Hyg 2010;82(5):930–6.
[7] Langevin S, Libman M, Drebot MA, Laverdière M. A case of Japanese encephalitis virus infection acquired during a trip in Thailand. J Travel Med 2012;19(2):127–9.

[8] Doti P, Castro P, Martínez MJ, Zboromyrska Y, Aldasoro E, Inciarte A, et al. A case of Japanese encephalitis in a 20 year-old Spanish sportsman, February 2013. Eurosurveillance 2013;18(35):20573.
[9] Kollaritsch H, Paulke-Korinek M, Dubischar-Kastner K. IC51 Japanese encephalitis vaccine. Expert Opin Biol Ther 2009;9(7):921–31.
[10] Jelinek T. IXIARO updated: overview of clinical trials and developments with the inactivated vaccine against Japanese encephalitis. Expert Rev Vaccines 2013;12(8):859–69.
[11] Rabe IB, Miller ER, Fischer M, Hills SL. Adverse events following vaccination with an inactivated, Vero cell culture-derived Japanese encephalitis vaccine in the United States, 2009–2012. Vaccine 2015;33(5):708–12.
[12] EPAR summary for the public. Ixiaro 2013, ⟨http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000963/human_med_000862.jsp&mid=WC0b01ac058001d124⟩; [accessed 12.02.15].
[13] Eder S, Dubischar-Kastner K, Firbas C, Jelinek T, Jilma B, Kaltenboeck A, et al. Long term immunity following a booster dose of the inactivated Japanese Encephalitis vaccine IXIARO®, IC51. Vaccine 2011;29:2607–12.
[14] Dubischar-Kastner K, Eder S, Buerger V, Gartner-Woelfl G, Kaltenboeck A, Schuller E, et al. Long-term immunity and immune response to a booster dose following vaccination with the inactivated Japanese encephalitis vaccine IXIARO, IC51. Vaccine 2010;28(32):5197–202.
[15] Hainz U, Jenewein B, Asch E, Pfeiffer KP, Berger P, Grubeck-Loebenstein B. Insufficient protection for healthy elderly adults by tetanus and TBE vaccines. Vaccine 2005;23(25):3232–5.
[16] Weinberger B, Grubeck-Loebenstein B. Vaccines for the elderly. Clin Microbiol Infect 2012;18(S5):100–8.
[17] Wagner A, Garner-Spitzer E, Jasinska J, Paulke-Korinek M, Hofer M, Kollaritsch H, et al. Characterisation of immune responses to primary Japanese encephalitis vaccination in elderly compared to young vaccinees. Poster at CISTM 2013 in Maastricht, PO 21.02.
[18] Dubischar-Kastner K, Cramer JP, Eder S, Jelinek T, Jilma B, Kollaritsch H, et al. Immunogenicity and safety of the inactivated Japanese encephalitis vaccine IXIARO®, IC51, in Elderly. Poster at CISTM 2013 in Maastricht, PO14.04.
[19] Stiasny K, Kiermayr S, Holzmann H, Heinz FX. Cryptic properties of a cluster of dominant flavivirus cross-reactive antigenic sites. J Virol 2006;80(19):9557–68.
[20] Crill WD, Chang GJ. Localization and characterization of flavivirus envelope glycoprotein cross-reactive epitopes. J Virol 2004;78(24):13975–86.
[21] Orlinger KK, Hofmeister Y, Fritz R, Holzer GW, Falkner FG, Unger B, et al. A tick-borne encephalitis vaccine based on the European prototype strain induces broadly reactive cross-neutralizing antibodies in humans. J Infect Dis 2011;203(11):1556–64.
[22] Mansfield KL, Horton DL, Johnson N, Li L, Barrett AD, Smith DJ, et al. Flavivirus induced antibody cross-reactivity. J Gen Virol 2011;92(Pt 12):2821–9.
[23] Schuller E, Klade CS, Heinz FX, Kollaritsch H, Rendi-Wagner P, Jilma B, et al. Effect of pre-existing anti-tick-borne encephalitis virus immunity on neutralising antibody response to the Vero cell-derived, inactivated Japanese encephalitis virus vaccine candidate IC51. Vaccine 2008;26(48):6151–6.
[24] Paulke-Korinek M, Kundi M, Laaber B, Brodtraeger N, Seidl-Friedrich C, Wiedermann U, et al. Factors associated with seroimmunity against tick borne encephalitis virus 10 years after booster vaccination. Vaccine 2013;31(9):1293–7.
[25] Garner-Spitzer E, Kundi M, Rendi-Wagner P, Winkler B, Wiedermann G, Holzmann H, et al. Correlation between humoral and cellular immune responses and the expression of the hepatitis A receptor HAVcr-1 on T cells after hepatitis A re-vaccination in high and low-responder vaccinees. Vaccine 2009;27(2):197–204.
[26] Garner-Spitzer E, Wagner A, Paulke-Korinek M, Kollaritsch H, Heinz FX, Redlberger-Fritz M, et al. Tick-borne encephalitis (TBE) and hepatitis B non-responders feature different immunologic mechanisms in response to TBE and influenza vaccination with involvement of regulatory T and B cells and IL-10. J Immunol 2013;191(5):2426–36.
[27] Zuckerman JN. Protective efficacy, immunotherapeutic potential, and safety of hepatitis B vaccines. J Med Virol 2006;78:169–77.
[28] Kubba AK, Taylor P, Graneek B, Strobel S. Non-responders to hepatitis B vaccination: a review. Commun Dis Public Health 2003;6:106–12.