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Investigation (In Vivo and In Vitro) of Booster Dose Vaccine Requirement for Long-Term Protection against Hepatitis B Virus Infection*

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Aim: Studies have shown that no booster dose was required at least 10 to 15 years after a primary vaccination for individuals who developed protective anti-hepatitis B surface (anti-HBs) antibodies. In this study, booster dose requirement for HBV after primary immunization was investigated.

Materials and Methods: Seventeen individuals vaccinated previously were enrolled in the study. They had once developed a protective level of anti-HBs antibody after immunization and their anti-HBs titer had declined to an underprotective level. Twenty uninfected and unvaccinated healthy people were chosen as controls. Lymphoproliferative response to in-vitro stimulation with hepatitis B surface antigen (HBsAg) and anti-HBs response to vaccine were evaluated for immune response.

Results: T lymphocytes from 4 (24%) of the study group showed lymphoproliferative response to HBsAg stimulation while none of the controls did ($P < 0.05$). In all subjects in the study group, anti-HBs response (≥10 mIU/ml) was detected 1 to 7 days after the booster injection but in only 2 of the controls antibody response was detected 28 days after the first dose of HBV vaccine ($P < 0.0001$).

Conclusions: A booster dose of HBV vaccine might not be required because of immunological memory.

Key Words: HBV vaccination, long-term protection, booster dose requirement, immunological memory

Hepatit B Virüsüne Karşı Aşılamada Uzun Süreli Korunma için Booster Doz Gerekliliğinin (İn Vivo ve İn Vitro) Araştırılması

Amaç: Çalışmalar; primer aşılamadan sonra antikor gelişen kişilerde en az 10-15 yıl süre ile booster doza ihtiyaç olmadığını göstermiştir. Bu çalışmada; primer aşılamadan sonra booster doza gereksinim araştırılmıştır.

Yöntem ve Gereç: Daha önce aşılanmış 17 kişi çalışmaya alındı. Bu kişilerde primer aşılamadan sonra anti-HBs oluşmuş ve zamanla anti-HBs titresi koruyucu düzeyin altına düşmüştü. Kontrol grubu olarak, enfekte olmamış ve aşılanmamış 20 kişi seçildi. Hücresel yanıtı araştırmak için, T lenfositlerin in-vitro HBsAg uyarısına karşı oluşturduğu lenfoproliferatif yanıt, ve aşı sonrası oluşan antikor yanıtı değerlendirildi.

Bulgular: Kontrol grubundakilerin hiçbirinde HBsAg uyarısına karşı yanıt görülmezken, çalışma grubundaki 4 kişide (% 24) T lenfositlerde lenfoproliferatif yanıt görüldü ($P < 0,05$). Çalışma grubunda booster dozdan 1-7 gün içinde antikor yanıtı oluşurken, kontrol grubundakilerin sadece ikisinde booster aşı dozu sonrası 28. günde antikor yanıtı oluştu ($P < 0,0001$).

Sonuç: İmmünolojik memori sayesinde booster HBV aşısı dozuna gereksinim olmayabilir.

Anahtar Sözcükler: HBV aşısı, uzun süreli korunma, booster doz gerekliliği

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Introduction

Hepatitis B virus (HBV) is the most frequent and the unique vaccine preventable cause of chronic viral hepatitis (1-5).

The standard application schedule for the HBV vaccine consists of 3 doses administered at 0, 1, and 6 months. For individuals at high risk of infection, another schedule consisting of 4 doses administered at 0, 1, 2, and 12 months is recommended (6-8).

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A higher anti-HBs antibody level after primary vaccination suggests a longer duration of protective immunity. The results of mass vaccination studies showed that most individuals (70% to 90%) responded well to primary vaccination, preserving their antibody at protective levels (10 mIU/ml) up to 10 to 12 years. Anti-HBs antibody level in some of the individuals vaccinated might decline to undetectable levels in 5 to 10 years. In such a situation, whether the protection continues or not is debatable. No case of clinical HBV infection among individuals to whom a primary vaccination schedule was applied has been reported, but there are a few reported cases of HBsAg carrier state (9-13).

Owing to immunological memory function, immune response to secondary immunization is more rapid and stronger than that to primary immunization (14). Therefore, periodic booster dose vaccine administration to individuals once fully vaccinated might not be required.

In this study, we aimed to show the immune response to HBV vaccine (in vivo and in vitro) in subjects vaccinated before and whose antibody levels declined below 10 mIU/ml and to determine if a periodic booster dose of vaccination was required.

Materials and Methods

This study was carried out in Gülhane Military Medical Academy (GMMA) Department of Infectious Diseases and Clinical Microbiology and Department of Immunology.

In 1997, serological screening (HBsAg, anti-HBc-IgG and anti-HBs) was performed by the Department of Infectious Diseases and Clinical Microbiology among health care workers (HCWs) working for GMMA training hospital. Records of 2184 individuals were reevaluated and we got in contact with subjects whose serological test results for HBV (HBsAg, anti-HBc IgG, and anti-HBs) were all negative. They were asked if they had been vaccinated with HBV vaccine and, if so, when and how many doses had been administered. They were also asked if they had anti-HBs antibody response. Seventeen individuals who were fully vaccinated, who developed anti-HBs antibody and whose anti-HBs antibody titer declined under 10 mIU/ml were enrolled in the study. Of these 17 individuals, 8 were male and 9 female.

Twenty healthy unvaccinated HCWs not exposed to HBV (i.e. negative for anti-HBc IgG) and whose age and sex were consistent with the study group were chosen as controls.

The study was designed as in vitro for cellular immune response evaluation and as in vivo for humoral immune response evaluation.

For in vitro cellular immune response evaluation, in sterile conditions 10 ml of heparinized venous blood was drawn from all individuals in both groups. The blood samples were centrifuged at 1500 rpm for 10 min and plasma from each sample was extracted. The same amounts of cell culture medium and plasma were mixed and then the mixture was layered on Histopaque (SIGMA, USA). After that, the mixture was centrifuged at 1500 rpm for 10 min. Interphase mononuclear cells were extracted and washed with 5 ml of cell culture medium 3 times. Amount of cells was standardized as 2×10^6 cells/ml and these cells were cultivated in a microplate with autologous plasma, 10^5 cells in each well. For investigating immune response to HBsAg, HBsAg was added until the final concentration reached 5 mg/ml. The cells were incubated for 72 h at 37 °C in an incubator containing 5% CO_2 and 95% dry air. Soon after, 0.5 mCi ^{3}H-Timidin (Amersham, UK) was added to each well and the plate was incubated for 18 h in the same incubator. After incubation, the cells were collected by a harvester and the amount of radioactive timidine was counted by a b-counter as counts per minute (cpm). The results of the counts were determined according to the mean of the counts of triple wells. The stimulation index was calculated by dividing the counts in medium with HBsAg and without HBsAg. The stimulation index higher than twice the standard deviation amount of the mean stimulation index of controls was considered positive for proliferative response.

For in vivo humoral immune response evaluation, 20 µg/ml of recombinant HBV vaccine (EUVAX B, North Korea) was administered to the deltoid region of all subjects intramuscularly. Blood was drawn from individuals in the study group 1, 3, 5, 7, 14, and 28 days after vaccination. Since anti-HBs response was not expected to develop in the first week of vaccination (15), blood was drawn from individuals in the control group only 7, 14, and 28 days after the first dose of vaccine was administered. Then the HBV vaccination schedule was completed in the control group. The blood drawn was centrifuged immediately and sera were stored at –85 °C until the anti-HBs assay was performed. The stored

samples were thawed and anti-HBs titers were detected by EIA (Abbott/Murex, Germany) by an automated EIA device (Tecan, Italy) according to the procedure suggested by the manufacturer. A titer less than 10 mIU/ml was considered negative, between 10 and 99 mIU/ml positive, and over 100 mIU/ml highly positive.

The number of subjects who developed protective antibodies was given as a percentage for the study group on days 1, 3, 5, 7, 14, and 28 after the booster dose of HBV vaccine, and for the control group on the days 7, 14, and 28 after the first dose of HBV vaccine. Statistical analysis was performed using Excel for Windows (Microsoft, USA).

Results

Of the 17 subjects in the study group (mean age: 30.47 ± 8.36 years and range: 18 to 47), 8 (47.05%) were male and 9 (52.95%) female. Of the control group (mean age: 30.2 ± 7.65 years and range: 18 to 45), 10 (50%) were male and 10 (50%) female. There was no significant difference between the study group and the control group in terms of their demographic characteristics (age, sex, obesity, body mass index, cigarette smoking and alcohol abuse, systemic illnesses, etc.) ($P > 0.05$).

In the in vitro investigation of cellular immunity, no lymphoproliferative response to HBsAg stimulation was observed in the control group but peripheral mononuclear cells from 4 (23.53%) individuals in the study group showed lymphoproliferative response ($P < 0.05$).

In the in vivo investigation of humoral immunity, just 24 h after the injection of booster HBV vaccine, protective anti-HBs antibody developed in 7 (41.17%) of the subjects, and on the other days the numbers of subjects who developed protective anti-HBs antibody were as follows: 10 (58.82%), 14 (82.35%), and 17 (100%) on days 3, 5, and 7, respectively.

The number of subjects who developed high protective (100 mIU/ml) anti-HBs antibody response was 2 (11.76%) on day 1 and was 3 (17.64%), 6 (35.29%), 8 (47.05%), 14 (82.35%), and 16 (91.11%) 3, 5, 7, 14, and 28 days after the booster dose of HBV vaccine, respectively.

No anti-HBs antibody response was observed in the control group in the first 2 weeks after the first vaccine. Only 2 (10%) of them had anti-HBs antibody on day 28.

There was a significant difference between the study and control groups in terms of their anti-HBs levels on day 28 of vaccination ($P < 0.0001$). The results of the in vivo investigation of humoral immunity are given in the Table.

Discussion and Conclusion

In 1981, plasma-derived HBV vaccine and, in 1996, recombinant HBV vaccine became commercially available. In those years, a routine booster dose of HBV vaccine was recommended in each 5-year period after the primary vaccination. However, nowadays, requirement for booster dose of HBV vaccine for life-long immunity is debated and it is suggested that even if a protective amount of anti-HBs antibody is not detected in serum samples of pre-vaccinated individuals, owing to immunological memory, protection still exist (16).

Table. The results of in vivo investigation of humoral immunity.

Days after HBV Vaccine	Subjects Developed Anti-HBs Antibody 10 mIU/ml		Subjects Developed Anti-HBs Antibody 100 mIU/ml	
	Study Group n / %	Control Group n / %	Study Group n / %	Control Group n / %
1	7 / 41.17	-	2 / 11.76	-
3	10 / 58.82	-	3 / 17.64	-
5	14 / 82.35	-	6 / 35.29	-
7	17 / 100	0 / 0	8 / 47.05	0 / 0
14	17 / 100	0 / 0	14 / 82.35	0 / 0
28	17 / 100	2 / 10	16 / 91.11	2 / 10

Both cellular immunity and humoral immunity play roles in immunological memory. However, most studies performed were based on B lymphocyte function evaluation. There are few studies based on T lymphocyte function evaluation.

In our study, we observed lymphoproliferative response in 24% (4/17) of the subjects in the study group. This result was lower than we expected but it was significantly higher than that in the control group ($P < 0.05$).

Huang et al. evaluated immunological memory in pre-vaccinated subjects comparing T cell lymphoproliferative response, IL-2 and IL-5 production of T lymphocytes, and anti-HBs antibody production of B lymphocytes before and a month after a booster dose of HBV vaccine. In their study, they detected T lymphocyte lymphoproliferative response in 47%, IL-2 response in 81%, and IL-5 response in 100% of the subjects, and, after the booster HBV vaccine injection, they detected T lymphocyte lymphoproliferative response in 52%, IL-2 response in 90%, and IL-5 response in 100% of subjects. Injection of HBV vaccine augmented T lymphocyte proliferative response and IL-2 production of T lymphocyte, but these increases were not significant. They also reported that IL-5 was the most predictive marker of immunological memory (17).

Leroux et al. activated T lymphocytes and observed their clonal proliferation. They reported that humoral immune response kinetics to antigenic stimulation was closely related to T cell response (18).

Nagaraju et al. observed lymphoproliferative response in only 3 (10.34%) of 29 pre-vaccinated cases but they detected sufficient amounts of antibody in all cases after a booster dose of HBV vaccine (19).

We also evaluated in vivo anti-HBs antibody response to a booster dose of HBV vaccine for B lymphocyte function and we achieved significant results. All subjects in the study group developed protective levels of anti-HBs antibody in the first 7 days but none in control group did until 14 days after the first vaccine ($P < 0.0001$). We suggested that this result was due to immunological memory.

Jilg et al. administered a booster dose of HBV vaccine and detected anti-HBs antibody level of 10 mIU/ml in 96% of 212 pre-vaccinated individuals who had responded to primary vaccination. They also detected anti-HBs antibody on days 3 to 5 of the booster injection in 4 individuals who had responded to primary vaccination but had no antibody detected at the time of booster injection. As a result, they reported immunological memory extended protection even if no antibody had been detected in individuals vaccinated before (20).

Krugman et al. (21), Milne et al. (22,23), and West et al. (13) reported the existence of immunological memory based on anti-HBs antibody response to a booster dose of HBV vaccine.

Boland et al. detected circulating B lymphocytes producing anti-HBs, using specific spot-ELISA test in 68% of 37 individuals whose antibody level was under 10 mIU/ml in 6-8 years after primary vaccination and they reported that protection against HBV continued also in individuals in whom anti-HBs antibody was not detected (24).

We used recombinant vaccine in our study. Because both plasma-derived and recombinant vaccines were immunogenic and effective (25-30), we did not compare the types of vaccine administered in primary vaccination. In addition, as vaccination schedule and dosage used in primary vaccination had no effect on immunological memory (6-8,17) we ignored these differences in our study.

Although the fact that anamnestic response develops in a very short time is encouraging, it is not possible to conclude there is no risk of infection until antibody develops, since the duration for HBV to reach hepatocytes is not well established.

Our study implied that immunological memory went on working in individuals whose anti-HBs antibody levels became undetectable and we concluded that primary HBV vaccination with no booster injection might be regarded as long-life preventive, but it is obvious that this prediction is valid for only immunocompetent and low-risk individuals.

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