Genetic polymorphisms of CXCR5 and CXCL13 are associated with non-responsiveness to the hepatitis B vaccine

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A B S T R A C T

A cohort based study has been undertaken to investigate the possible association of genetic polymorphisms in genes functionally related to follicular T helper (THF) cells with non-responsiveness to hepatitis B virus (HBV) vaccination. A total of 24 single nucleotide polymorphisms (SNPs) in 6 THF related genes (CXCR5, ICOS, CXCL13, IL-21, BCL6 and CD40L) were investigated in 20 non-responders and 45 responders to HBV vaccination. Genetic association analysis revealed that three SNPs (rs497916, rs3922, rs676925) in CXCR5 and one SNP (rs355687) in CXCL13 were associated with hepatitis B vaccine efficacy. In addition, significantly unbalanced distributions of two haplotypes, defined by three SNPs (rs497916, rs3922, rs676925) within CXCR5, were also seen between non-responders and responders. Furthermore, we demonstrated that the rs3922 “GG” genotype was associated with higher levels of CXCR5 than the “AG” and “AA” genotype in a group of healthy volunteers. A dual luciferase report assay was used to confirm that the “G” allele in rs3922 may lead to higher gene expression than the “A” allele, implicating that rs3922 might be a functional SNP affecting CXCR5 expression. These results indicated that polymorphism associated changes in CXCR5 expression in THF cells may be associated with non-responsiveness to hepatitis B vaccination.

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1. Introduction

Hepatitis B vaccines have an outstanding record of safety and effectiveness. However, a small minority of vaccinees, so-called non-responders, produce an inadequate neutralizing antibody response following receipt of the standard vaccination regime and are therefore probably still susceptible to infection with hepatitis B virus (HBV) [1,2]. In addition to a number of technical factors such as the intervals between the administration of vaccine, doses administered and specific vaccine formulation, a number of reports have suggested that vaccinee specific variations such as age, male gender, obesity, smoking, chronic disease, immunodeficiency and crucially genetic predisposition may also be involved in low or null responses to HBV vaccines [3–8]. In recent years, an increasing number of reports have linked specific genetic polymorphisms of immune system markers such as IL-1β, IL-2, IL-4, IL-10, IL-4RA, IL-13 and TLR-2 with non-responsiveness to HBV vaccine [4,9,10].

It is universally recognized that most vaccines in current use are able to induce the synthesis of long-lived protective antibodies from memory B cells and plasma cells. Help from specific CD4+ subsets of T cells to B cells is a prerequisite for this humoral immunity. Follicular T helper (THF) cells are a newly recognized lineage of CD4+ T cells [11], that were originally discovered in the B cell follicles of secondary lymphoid organs with the defining feature of high expression of the chemokine receptor CXCR5. There are accumulating evidences that these THF cells are the key T-cell subset required...
for the formation of germinal centers (GCs) and the generation of antigen specific T cell-dependent antibody responses [11–15].

That Th17 cells are actively engaged in responses to vaccination has been shown in a number of different virus systems. Bentebele et al. reported that peripheral Th17-like cells, marked as CD4+ ICOS+ CXCR3+ CXCR5+, are associated with protective antibody responses after seasonal flu vaccination [16]. The efficacy of the foot and mouth disease vaccine (FMDV) may also be enhanced through the generation of Th17 cells [17,18]. Furthermore, the nonresponsiveness of HIV-infected individuals to the 2009 H1N1 vaccine has been primarily attributed to the impairment of circulating Th17 cells [19]. In the case of HBV, the abnormal expressions of Th17-related molecules have been reported to be at least partially responsible for the dysfunction of immune responses during chronic HBV infection [20,21].

Despite this clear evidence that Th17 cells have an important role in the humoral immune response to a number of vaccines, the relationship between Th17 cells and specific antibody responses to HBV vaccine has not as yet received sufficient attention. Given the growing recognition of the importance of Th17 cells in generating a strong humoral immune response, it seems reasonable to hypothesize that polymorphisms of Th17 related molecules may be associated with nonresponsiveness to HBV vaccination. Therefore, in this study a total of 24 single nucleotide polymorphisms (SNPs) within six genes (CXCR5, ICOS, CXCL13, IL-21, BCL6 and CD40L) were selected and analyzed.

2. Materials and methods

2.1. Study cohort

The cohort recruited for the current study was a subset from a previous survey on non-responders to HBV vaccine [4,22]. The details for screening were described in Supplementary Fig. 1. In brief, a total of 37,221 ethnic Han Chinese volunteers with no hepatitis B vaccination history were recruited. All recruited volunteers were vaccinated with 10 μg of recombinant HBV vaccine (Shenzhen Kangtai Biological Products Co., Ltd., Shenzhen, Guangdong) according to the standard 0, 1, and 6 months vaccination schedule. Anti-HBs titers were tested at 7th month after initiating the vaccination regime and individuals whose anti-HBs titer was lower than 10 mIU/ml were re-vaccinated with a further 3 doses of HBV. Levels of Anti-HBs antibody were re-tested approximately one month after the final dose of vaccine was administered. For the present study, volunteers with either evidence of previous HBV infection or deemed to be suffering from an occult HBV infection were excluded. More screening criteria were listed in Supplementary Fig. 1. At the end of this process 26 individuals from the cohort recruited were defined as authentic non-responders based on producing anti-HBs levels of less than 10 mIU/ml after having received a total of six doses of vaccine administered over two consecutive rounds of vaccination schedule. DNA samples from 20 of these non-responders were available for use in this study. For comparative purpose, after considering almost the same criteria for screening non-responders, a group of vaccine responders were identified on the basis of having produced anti-HBs levels equal to or more than 100 mIU/ml after having received the standard 3 doses of vaccine. Finally 45 responders were randomly selected and there are no significant differences between the responders and non-responders in age (age range 25–60 for responders vs. age range 30–59 for non-responders, P = 0.0512) and gender (23F/22M for responders vs. 7F/13M for non-responders, P = 0.2291). The detailed demographic data of the 20 non-responders and 45 responders is shown in Supplementary Table 1. Since no peripheral blood mononuclear cells (PBMC) were available from the non-responders and responders, 29 healthy adults who had physical examination in Peking University Third Hospital without evidence of prior HBV infection were also enrolled for further experiments. This study was approved by the Ethics Committee of the Peking University Health Science Center and all subjects provided signed informed consent.

2.2. SNP selection and detection

Six Th17 associated molecules CXCR5, ICOS, CXCL13, IL-21, BCL6 and CD40L were selected for SNP analysis. Altogether 24 SNPs within these genes were chosen for the analysis (Supplementary Table 2), according to the following 2 criteria: first, the minor allele frequency (MAF) obtained from NCBI SNP database (http://www.ncbi.nlm.nih.gov/SNP/) or the SNP browser software 4.0 (Applied Biosystems) should be higher than 10% in the ethnic Han Chinese population. Second, there should be published evidence showing that the SNP is associated with some disease. Genomic DNA extracted as previously described was dissolved in sterile double distilled water and stored at −20 °C [4]. SNP genotyping was undertaken by Bioyong Technology using Sequenom MassARRAY technology (Bioyong Technology Co., Beijing, China).

2.3. Flow cytometry assays

Peripheral Blood Mononuclear Cells were isolated using Histopaque-1077 (Sigma, 10771) according to the manufacturer’s instructions and stored at −80°C. For flow cytometry assays, recovered cells were incubated for 30 min with a cocktail of antibodies that included eFluor450 conjugated anti-CD3 mAb (eBioscience, 48–0038), PE-Cy7 conjugated anti-CD4 mAb (BD, 557852), APC conjugated anti-CD19 mAb (BD, 555415) and PE conjugated anti-CXCR5 mAb (eBioscience, 12–9185). Following incubation the cells were washed with PBS and fixed with 2% paraformaldehyde. Samples were detected using a BD LSRIIFortessa flow cytometer and analyzed using FlowJo software.

2.4. Construction of vectors

To construct the miR-558 expression plasmid, a precursor of the miR-558 sequence amplified from HepG2 genomic DNA was cloned into the pRNA-U6.1/Neo-siFluc vector. The 3’-UTR region of CXCR5 including the rs3922 locus was amplified and inserted downstream of the luciferase reporter gene in pGL3-Control Vector. The luciferase reporter plasmid carrying an “A” allele in rs3922 was marked as pGL3-3922A-luc, while the pGL3-3922G-luc contains the SNP “G”.

2.5. Dual-luciferase reporter assays

HEK 293T cells were seeded into 12-well plates. Twenty-four hours later, the cells were co-transfected with 1.5 μg of miR-558 expression plasmid or U6 control vector and 50 ng pGL3-3922 luciferase vectors. The pRL-TK(25 ng) plasmid was also transfected as a transfection efficiency control. The luciferase activity in each well was quantified 24 h after transfection using a dual luciferase reporter kit (Promega, Madison, WI, USA) according to the manufacturer’s instructions. In an additional experiment only luciferase vector (pGL3-3922A-luc or pGL3-3922G-luc) and pRL-TK plasmid were co-transfected, without the miR-558 expression plasmid or U6 control vectors. The majority of most experimental conditions were the same in this case except the quantity of pGL3-3922 vector added was 100 ng per well.
Table 1
Association of TH1 gene SNPs with non-responsiveness to the HBV vaccine (a P-value < 0.05 is shown in bold and italics).

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Genotype and allele type</th>
<th>Non-responders (%)</th>
<th>Responders (%)</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCR5</td>
<td>rs3922</td>
<td>AA</td>
<td>5 (25)</td>
<td>23 (56.1)</td>
<td>0.26 (0.08–0.85)</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>3 (15)</td>
<td>4 (9.76)</td>
<td>1.63 (0.33–8.11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AG</td>
<td>12 (60)</td>
<td>14 (31.5)</td>
<td>3.12 (1.02–9.48)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A allele</td>
<td>22 (55)</td>
<td>60 (73.17)</td>
<td>0.45 (0.20–0.99)</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G allele</td>
<td>18 (45)</td>
<td>22 (26.83)</td>
<td>2.23 (1.01–4.92)</td>
<td></td>
</tr>
<tr>
<td>CXCR5</td>
<td>rs676925</td>
<td>GG</td>
<td>2 (10)</td>
<td>1 (2.27)</td>
<td>4.78 (0.41–56.07)</td>
<td>0.100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>11 (55)</td>
<td>34 (77.27)</td>
<td>0.36 (0.12–1.11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CG</td>
<td>7 (35)</td>
<td>9 (20.45)</td>
<td>2.09 (0.65–6.78)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G allele</td>
<td>11 (27.5)</td>
<td>11 (25.5)</td>
<td>2.66 (1.04–6.79)</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C allele</td>
<td>28 (72.5)</td>
<td>77 (87.5)</td>
<td>0.38 (0.15–0.96)</td>
<td></td>
</tr>
<tr>
<td>CXCR5</td>
<td>rs497916</td>
<td>TT</td>
<td>3 (15)</td>
<td>2 (4.55)</td>
<td>3.71 (0.57–24.18)</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>7 (35)</td>
<td>30 (68.18)</td>
<td>0.25 (0.08–0.77)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT</td>
<td>10 (50)</td>
<td>12 (27.27)</td>
<td>2.67 (0.89–8.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>T allele</td>
<td>16 (40)</td>
<td>16 (18.18)</td>
<td>3.00 (1.30–6.90)</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C allele</td>
<td>24 (60)</td>
<td>72 (81.82)</td>
<td>0.33 (0.14–0.77)</td>
<td></td>
</tr>
<tr>
<td>CXCL13</td>
<td>rs355687</td>
<td>TT</td>
<td>10 (52.6)</td>
<td>18 (40.91)</td>
<td>1.60 (0.54–4.74)</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>4 (21.1)</td>
<td>2 (4.55)</td>
<td>5.60 (0.93–33.77)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC</td>
<td>5 (26.3)</td>
<td>24 (54.55)</td>
<td>0.30 (0.09–0.97)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>T allele</td>
<td>25 (65.8)</td>
<td>60 (68.18)</td>
<td>0.90 (0.40–2.01)</td>
<td>0.793</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C allele</td>
<td>13 (34.2)</td>
<td>28 (31.82)</td>
<td>1.11 (0.50–2.50)</td>
<td></td>
</tr>
</tbody>
</table>

2.6. Statistical analysis

For each SNP, the association between response statuses to HBV vaccine and various genotypes or allele types was estimated by the chi-square test using SAS version 9.1.3 (SAS Institute, Inc., Cary, NC, USA). The Hardy–Weinberg equilibrium (H–W equilibrium) was calculated based on the control group using Haploview version 4.2 software [23]. Linkage disequilibrium (LD) analysis and haplotype construction were carried out with the same software. Specific parameters were set as previously published [4]. P-values, odds ratios (OR), and 95% confidence intervals (95% CIs) were obtained for correlation analysis. A P-value < 0.05 was taken to be statistically significant.

3. Results

3.1. Associations between TH1 gene SNPs and non-responsiveness to HBV vaccination

A total of 24 SNPs from TH1 associated molecules were analyzed in the 20 non-responders and 45 responders. The genotype and allele frequencies of all the SNPs in the study and control groups are listed in Supplementary Table 3. The H–W equilibrium was evaluated in the normal response group and two SNPs (rs3092945, rs715762) in CD40L were excluded from the analysis due to disequilibrium (P < 0.001).

Of the remaining 22 SNPs, four (rs3922, rs676925, rs497916 and rs355687) showed significant associations with the immune response triggered by HBV vaccination (P < 0.05, Table 1). Three of these were located in the CXCR5 gene: rs3922 (in 3′-UTR), rs676925 (in 3′-UTR) and rs497916 (in intron), while the fourth one rs355687 was located in the intron of CXCL13. As collected by the international Hapmap project, the distributions of these 4 SNPs in different populations were summarized in the Supplementary Table 4. What’s worth mentioning, the genotype and allele frequencies in our responder cohort are almost the same as that among the Han Chinese in Beijing, China (CHB), as well as the Chinese in Metropolitan Denver, Colorado (CHD) from the Hapmap project ([http://hapmap.ncbi.nlm.nih.gov/](http://hapmap.ncbi.nlm.nih.gov/)). As shown in Table 1, the ‘G’ allele frequency of rs3922 was significantly higher in non-responders than those normally responded to HBV vaccination (45% vs. 26.83%, P = 0.045). Consequently carriers of the ‘G’ allele at rs3922 site had an increased risk of failing to respond to HBV vaccination than those carrying the ‘A’ allele (OR = 2.23, 95% CI 1.01–4.92). Similarly, the minor allele ‘C’ in rs676925 increased the risk of non-response to vaccination (OR = 2.66, 95% CI 1.04–6.79, P = 0.037). In the case of rs497916, both the allele type and genotype were related with HBV vaccine efficacy (allele type: P = 0.008, genotype: P = 0.023). The ‘C’ allele in rs497916 protected from non-response (OR = 0.33, 95% CI 0.14–0.77) and the genotypes ‘TT’ and ‘CT’ increased the possibility of non-response to vaccination (‘TT’: OR = 3.71, 95% CI 0.57–24.18, ‘CT’: OR = 2.67, 95% CI 0.89–8.01). Finally, the ‘TC’ genotype in rs355687 appears more frequently in the group defined as HBV responders (P = 0.038, OR = 0.30, 95% CI 0.09–0.97).

3.2. Association of SNPs haplotypes and non-responsiveness to HBV vaccination

Using the Haploviev software, three possible blocks were constructed (Fig. 1). Strong linkage disequilibrium was found in two haplotypes in block one which was made up of rs497916, rs3922 and rs676925 within CXCR5. Compared to HBV vaccination responders, the ‘CAC’ haplotype had a significantly lower frequency in non-responders (Responders vs. non-responders: 0.735 vs. 0.513, P = 0.013). The frequency of the ‘TGG’ haplotype was 0.266 in the study group and only 0.111 in the control group (P = 0.025). That is, an individual who has a ‘TGG’ haplotype containing the three risk alleles of rs497916, rs3922 and rs676925 is significantly more likely to have nonresponsiveness to HBV vaccination.

3.3. The rs3922 affects CXCR5 expression on circulating CD4+ T and CD19+ B cells

Changes in the SNP located in the 3′-UTR may cause a fluctuation in gene expression. To understand whether the 2 chosen SNPs (rs3922, rs676925) that fall in the 3′-UTR of CXCR5 affected gene’s expression levels, flow cytometry assays were performed to detect CXCR5+ populations in PBMCs from 29 healthy individuals. Based on their genotypes in rs3922 or rs676925, this cohort was divided into 3 groups. The percentage of CXCR5 positive cells and the mean fluorescence intensity (MFI) of CXCR5 in CD3+CD4+ T cell and CD3–CD19+ B cell populations were compared amongst these 3 groups. The gating strategy employed is defined in Fig. 2A. As summarized in Fig. 2B, in both CD4+CD3+ T cell and CD19−CD3− B cell populations, the percentage and MFI values for CXCR5+ cells
in the rs3922 "GG" genotype group were significantly higher than those seen for the "AG" group (P < 0.05). Merging the data from both the "AA" group and "AG" group, still resulted in a statistical difference (P < 0.05), implying that the percentage of CXCR5+ cells is higher in the "GC" group than in the "non-GC" group. The same conclusion was true for the MFI value of CXCR5. However, no significant difference was observed when similar analysis was carried out on rs676925 (Supplementary Fig. 2). These results suggested that rs3922 might be involved in non-responsiveness to HBV vaccination through affecting the level of CXCR5 expression.

3.4. Experiments in vitro confirm the influence of rs3922 on CXCR5 gene expression.

Targetscan (http://www.targetscan.org/) prediction suggested that the rs3922 SNP is located in a potential microRNA binding site for miR-558 when the A allele is present, but not the G allele. To investigate whether allelic change in rs3922 can result in miR-558 regulated differences in the expression of CXCR5, luciferase vectors pGL3-3922A-luc and pGL3-3922G-luc differing only in the allelic version of the potential miRNA binding site were constructed (Fig. 3A). These luciferase vectors were independently co-transfected into HEK293T cells together with either miR-558 expressing or U6 control plasmids. Strikingly, cells co-transfected with pGL3-3922A-luc produced significantly lower luciferase activity than those co-transfected with pGL3-3922G-luc irrespective of whether the co-transfection was with the U6 control plasmid or that expressing miR-558 (Fig. 3B). Similarly, when only the luciferase reporter vector alone was transfected into cells, the lowest relative level of luciferase activity was recorded from pGL3-3922A-luc and the difference between the level of luciferase expressed by the pGL3-3922A-luc and that by the pGL3-3922G-luc was statistically significant (Fig. 3C).

4. Discussion

The standard HBV vaccination regime provides protection from HBV infection in most vaccinees, leaving only 5–10% of recipients defined as non-responders. A variety of factors, including gene polymorphisms, have been found to cause inadequate antibody production and hence limit the efficacy of the HBV vaccine [4,24]. Following the recognition that TfH cells play an important role in antibody responses, this study focused on the genes encoding 6 molecules associated with TH cells (CXCR5, CXCL13, ICOS, CD40L, IL-21 and BCL6), to evaluate possible associations of polymorphisms in them with immune responses made to HBV vaccination.

This SNP based association analysis clearly showed that polymorphisms in CXCR5 and CXCL13 were associated with non-responsiveness to the HBV vaccine. CXCR5 and CXCL13 appear to be inter-related not only in terms of anatomical location, but also in terms of the functioning of TH cells [25]. These two molecules are expressed both by TH cells and B cells [26,27]. The encounter between a CD4+ helper T cell and a cognate B cell is essential for TH cells to offer help in the production of antibody by B cells and it has been suggested that proper interplay between CXCR5 and CXCL13 is the impetus for TH cells and B cells to migrate to B cell follicles [28]. Consequently, changes in the expression level of these two molecules may affect the interaction between TH and B cells and subsequently the humoral response to vaccination. Annamalai and Selvaraj have reported in birds that following receipt of a coccidial vaccine, the miRNA level of CXCR5 in some specific organs increased substantially [29]. Also Guo et al. have shown that fusion of a vaccine antigen directly to CXCL13 could enhance DNA vaccine potency [30]. Thus, the linkage of CXCR5, CXCL13 polymorphisms to HBV vaccine efficacy is consistent with these other studies indicating that TH cells played a critical role in antibody production. The majority of previous studies have suggested that circulating CXCR5+CD4+ T cells have the essential features similar to the TH cells from lymphoid organs [31,32]. So we compared the CXCR5 positive populations in CD3+CD4+ T cells or CD3+CD19+ B cells in peripheral blood from different genotype populations. In an attempt to demonstrate an association between the SNPs in the 3′-UTR (rs3922 and rs676925) and gene expression level, 29 healthy volunteers were recruited and genotyped. This was necessary because of the paucity of RNA or PBMCs from the responders and non-responders to HBV vaccination making up the study cohort. Individuals with rs3922 “GG” genotype had a higher CXCR5 expression level in the blood than “non-GG” groups. This observation was concordant with our luciferase assays and hence the data suggested that “G” allele may correlate with a relative high gene expression. In the current study, a role for miR-558 was excluded and the detailed mechanism by which the “G” allele favors CXCR5 gene expression remains unknown.

It appears counter-intuitive that the “G” allele, which is associated with the non-respondor phenotype, should correspond to a higher expression of CXCR5. However, it remains unclear whether higher CXCR5 expression on TH cells will enhance their B cell help function. In fact, Bentebibel et al. have reported that, in human
Fig. 2. The characteristics of CXCR5 expression in circulating total CD4+ T cells and B cells were compared among different genotypes of rs3922 in 29 healthy controls. (A) Defining the gating strategy, CXCR5+ populations were derived from CD4+CD3+ or CD19+CD3− lymphocytes. (B) Whether in the CD4+CD3+ T cells or in the CD19+CD3− B cells, the percentage and MFI of CXCR5+ population were significantly higher in the group with “GG” genotype in rs3922 than in the “AG” group or in the “non-GG” group (P<0.05).
tonsils, the CD4+ subset (CXCR5hiCD4+) expressing low levels of CXCR5 secreted more IL-21 and IL-10 than the high expression subset (CXCR5hi). They also appeared to provide more efficient help for the differentiation of naive B cells into Ig-producing cells outside the germinal center [33].

Overall, this study supports the idea that polymorphisms in CXCR5 and CXCL13, two of TFH associated genes, are closely related to the non-responsiveness to HBV vaccination. The restricted number of non-responsive individuals in our cohort population and the consequent limitation in the availability of blood samples precluded further investigation of how the polymorphisms in CXCR5 and CXCL13 might affect the functioning of these genes. Therefore, how the expression levels of these genes can affect the efficacy of HBV vaccination is still a puzzle. However, achieving a better understanding of the functions of CXCR5 and CXCL13, particularly in response to HBV vaccination, may provide clues that can facilitate optimization of HBV vaccines.

Acknowledgements

We are grateful to the individuals who participated in this study and to the Center for Disease Control and Prevention in Jiangsu Province for sample collection. This work was supported by grants from the National S\&T Major Project for Infectious Diseases (2013ZX10002002 and 2012ZX10002001), the National Natural Science Foundation of China (81271826), the Natural Science Foundation of Beijing (7122108), the 111 Project (B07001).

Conflict of interest

The authors have no conflict of interest to declare.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.07.064.

References


Annamalai T, Selvaraj RK. Chemokine receptor CCR7 and CXCR5 mRNA in chickens following inflammation or vaccination. Poult Sci 2011;90(8):1695–700.


