Influenza vaccination of dialysis patients: cross-reactivity of induced haemagglutination-inhibiting antibodies to H3N2 subtype antigenic variants is comparable with the response of naturally infected young healthy adults

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Abstract

Background. Annual influenza vaccination is recommended for patients with chronic renal failure, although vaccination responses in haemodialysis (HD) patients may be suboptimal. Typically, the seroreactivity has been analysed against the vaccine virus or the corresponding year’s epidemic virus. No studies analysing cross-reactivity against subsequent years’ viruses have been presented.

Methods. Twenty-three chronic HD patients and 26 cardiac patients were, in autumn 1995, vaccinated with a trivalent influenza vaccine. The cross-reacting haemagglutination-inhibiting antibodies to five consecutive years’ (the last season 1999–2000) drift variants of H3N2 subtype influenza A virus were measured and compared with those of vaccinated cardiac patients and with those of 26 healthy military conscripts who suffered a serologically confirmed influenza A infection in the season 1995–1996.

Results. The influenza vaccination in HD patients resulted in comparable cross-reacting antibodies to the antibodies induced both by vaccination in cardiac patients and by natural infection in military conscripts. After a steady decline, the cross-reactivity to the latest epidemic virus improved in all the groups. This may be due to two reverted amino acid changes in the HA1 domain of the virus haemagglutinin.

Conclusions. Influenza vaccination in HD patients is as effective as the vaccination of cardiac patients with normal kidney function. The cross-reactivity of vaccination-induced antibodies is even as good as that of antibodies induced by natural infection of young healthy males. Additionally, vaccination seems to prime the individual beneficially against subsequent years’ influenza viruses.

Keywords: cross-reactivity; haemodialysis; influenza; vaccination; vitamin D

Introduction

Influenza vaccination is a safe and relatively effective way to prevent morbidity and mortality [1]. Uraemic patients are especially vulnerable to infections [2] and it is generally recommended to vaccinate patients with chronic renal insufficiency yearly against influenza [3]. In patients on haemodialysis (HD) the influenza vaccination response has been considered suboptimal [4], but recent studies, including our own, have shown almost comparable responses with healthy controls [5,6].

Influenza vaccination may be less prone in stimulating serum antibodies of high avidity than natural infection [7]. Additionally, the antigenic match between the vaccine viruses and the actual epidemic viruses is frequently incomplete. The protective efficacy of the vaccine is, however, dependent on its ability to provoke cross-reacting antibodies to the actual epidemic viruses.

Homologous serum antibody responses to the H3N2 vaccine virus and the cross-reactivity of the induced antibodies to the epidemic virus were recently shown to decline with the increasing age of the people being vaccinated [8], and T-cell dependent B-cell functions, for example influenza vaccine response, are compromised in uraemia [9]. These facts raise a concern about the cross-reactivity of the antibodies induced by the vaccination of HD patients. In the present study we
measured the cross-reactivity of antibodies induced by influenza vaccination in HD patients. This was, in addition to the corresponding year’s epidemic virus, also done for the five following seasons’ drift variants of H3N2 subtype epidemic influenza A virus. This is the first time the effect of influenza vaccination on the formation of cross-reacting antibodies to subsequent years’ drift virus variants has been studied in HD patients.

Subjects and methods

The HD and cardiac patients were part of a larger study group, of which the basic vaccination results have been reported previously [5]. Twenty-three HD patients (mean age 55 years, six women, 17 men) dialysed (mean time in HD treatment 24 months, range 1–55 months) with synthetic biocompatible dialysis membranes, and 26 patients (mean age 63 years, 10 women, 16 men) from a cardiac ward who had a normal kidney function and received no immunosuppressive therapies, were, in the autumn of 1995, vaccinated with a commercially available inactivated trivalent vaccine (Vaxigrip®; Pasteur Merieux Serums et Vaccins). The vaccine contained 15 μg of antigen from the component strains A/Johannesburg/33/94 (H3N2), A/Texas/36/91 (H1N1) and B/Harbin/7/94. Almost all HD patients (20/23; 87%) had previously received influenza vaccinations: four patients before HD treatment and 16 while on HD. Only two (8%) cardiac patients had been vaccinated previously.

The blood specimens were collected at the time of vaccination (pre-vaccination sample) and 5 weeks thereafter (post-vaccination sample). Paired sera collected during the 1995–1996 epidemic season from 26 healthy male military conscripts (age range 18–24 years) in the acute and convalescent phases of serologically confirmed (a 4-fold or greater antibody rise in a standard complement-fixation antibody test) influenza A infections were at our disposal. The military conscripts had not been vaccinated during that season. All the sera were stored at −20°C until studied for haemagglutination-inhibiting (HI) antibodies in autumn 2000.

HI assay was performed as outlined previously [10], using goose erythrocytes instead those of hens. The H3N2 subtype vaccine virus A/Johannesburg/33/94 (JHN/33/94) and five virus strains isolated during the five epidemic seasons since 1995–1996 in Finland served as antigens in the HI tests. The drift variants were isolated and cultivated exclusively in MDCK cell cultures and the vaccine strain in embryonated eggs. Antigenic relationships of the vaccine virus (JHN/33/94) and epidemic viruses were analysed in the HI tests using rat antisera as described previously [11].

The vaccine virus and the five drift variants were studied for their nucleotide sequences coding for the variable HA1 domain of the virus haemagglutinin. Detailed techniques (RNA extraction, cDNA synthesis, amplification procedures with PCR and sequencing) have been described previously [12]. Four virus strains were sequenced for the present study: JHN/33/94, Finland/381/95, Finland/680/99 and Finland/749/00. Two strains were sequenced previously: Finland/539/97 and Finland/579/98.

The statistical significances of the differences in influenza A antibody levels among and between the three groups were calculated with the Kruskall–Wallis test. A P<0.05 was considered significant. The statistics were calculated using the SPSS statistical software (SPSS Inc., USA).

All the patients gave their informed consents and the study was approved by the local ethical committee.

Results

Table 1 illustrates the antigenic relationship of the vaccine virus strain JHN/33/94 and the drift variants isolated in Finland during the five consecutive seasons from 1995–1996 to 1999–2000. The antisera produced against the vaccine strain (JHN/33/94) reacted to epidemic virus strains isolated in years 1995–1998, but not to those viruses isolated in 1999 and 2000 (FN680/99, FN749/00). The antisera to the epidemic strains of 1995–1996 (FN381/95) and 1997–1998 (FN/539/97) reacted at low titres both with the vaccine strain of 1995 (JHN/33/94) and with all the drift variants, even with the most recently isolated one (FN/749/00). The antisera to the epidemic strains after the season 1997–1998 (FN/579/98, FN/680/99, FN/749/00) did not react with the vaccine virus of the year 1995 (JHN/33/94).

Amino acid changes in the variable HA1 domain of virus haemagglutinin are listed in Table 2. Twelve amino acid substitutions in HA1 differentiated the vaccine virus (JHN/33/94) from the corresponding year’s epidemic virus FN/381/95. The number of amino acid substitutions of the following successive epidemic virus variants increased gradually to 19, 22, 24 and 30.

<table>
<thead>
<tr>
<th>Virus strains</th>
<th>HI titers of rat antisera against</th>
</tr>
</thead>
<tbody>
<tr>
<td>JHN/33/94</td>
<td>320</td>
</tr>
<tr>
<td>FN/381/95</td>
<td>640</td>
</tr>
<tr>
<td>FN/539/97</td>
<td>320</td>
</tr>
<tr>
<td>FN/579/98</td>
<td>20</td>
</tr>
<tr>
<td>FN/680/99</td>
<td>&lt;10</td>
</tr>
<tr>
<td>FN/749/00</td>
<td>&lt;10</td>
</tr>
<tr>
<td>FN/381/95</td>
<td>80</td>
</tr>
<tr>
<td>FN/539/97</td>
<td>160</td>
</tr>
<tr>
<td>FN/579/98</td>
<td>160</td>
</tr>
<tr>
<td>FN/680/99</td>
<td>160</td>
</tr>
<tr>
<td>FN/749/00</td>
<td>160</td>
</tr>
</tbody>
</table>

Homologous titres are shown in bold.
Table 2. Amino acid differences in the HA1 domain of the virus haemagglutinin between the vaccine virus of autumn 1995 (JHN/33/94) and five successive drift variants from the epidemic seasons 1995–1996 to 1999–2000

<table>
<thead>
<tr>
<th></th>
<th>FN/381/95</th>
<th>FN/539/97</th>
<th>FN/579/98</th>
<th>FN/680/99</th>
<th>FN/749/00</th>
</tr>
</thead>
<tbody>
<tr>
<td>JHN/33/94</td>
<td>GQSPRKNINNVDKVYGNKTVGTQYQRGNDGNSD</td>
<td>G.S.Q.TKKG.T...K...D...QNSVSS.D.N.</td>
<td>G.E...N...SRTQKRAD.AQSNSVSS.KN.</td>
<td>VH.FSQET.N.SINTSR.KCK.EIAAQNSVSS.KN.</td>
<td>VN.MQETN.SINTSR.KCK.EIAAQNSVSS.KN.</td>
</tr>
</tbody>
</table>

The most recent virus variant exhibited two reverted amino acid changes at residues 144 and 160.

Although the most recent variant (FN/749/00) had more substitutions than the preceding years’ variants, it exhibited two reverted amino acid changes (at residues 144 and 160 amino acids changed to those seen in the vaccine virus).

Table 3 shows the cross-reacting HI antibodies induced by the vaccinations of the HD and the cardiac patients in autumn 1995 and induced by influenza A infections of the conscripts in winter 1995–1996. The pre-vaccination antibody levels of the HD patients to each of the five epidemic virus strains were higher than those of the cardiac patients and the military conscripts. The proportion of the HD patients who exhibited protective antibody titres to the epidemic virus FN/381/95 rose from 35 to 78% (18 23) by vaccination, which is comparable (73 and 77%, respectively) with the response induced by vaccination in the cardiac patients and after natural infection in the military conscripts. The mean fold increase of HI antibody titres of the HD patients amounted to 3.3 (from 1.27 to 1.77 in log values). The corresponding mean fold increase of the cardiac patients and the conscripts were 11 and 8, respectively.

Cross-reactivity of the antibodies induced by vaccinations and natural infections decreased gradually when epidemic virus variants from the seasons 1995–1996 to 1998–1999 were used as antigens in HI tests (Table 3). This decrease is consistent with the antigenic relationship of the drift variants demonstrated using rat antisera in HI tests (Table 1). After the decrease, the cross-reactivity enhanced in all study groups, which is seen as the increased rate of protective titres in the post-vaccination and convalescent phases. The vaccination induced a substantial increase in HD patients’ antibody levels, as the post-vaccination antibody levels were significantly higher than the pre-vaccination ones against all studied virus variants except that of year 1998–1999 (FN/680/99). The significance levels were in chronological order: $P = 0.002$, $P = 0.003$, $P = 0.014$, $P = 0.11$ and $P = 0.03$.

Discussion

Vaccination-induced antibody response is a clinically relevant way to study immune functions, as it reflects both antigen recognition and processing and humoral response in a real life situation. The vaccination of HD patients in 1995 proved to be effective in provoking HI antibodies to the actual epidemic H3N2 subtype influenza virus of the following season (MDCK-grown FN/381/95). The post-vaccination protection rate (78%) reached even a higher value than previously detected for the vaccine virus strain, the egg-grown JHN/33/94 (36%) [5]. This is not surprising, for MDCK-grown virus variants correspond better than the egg-grown variants to the virus excreted by human host [13,14]. In the HD patients the protection rate and the mean fold increase of HI antibody titres were above the requirements of the Committee for Proprietary Medicinal Products (CPMP 1997) [15].

In HD patients the mean fold increase of HI antibody titres was smaller than in cardiac patients and in naturally infected conscripts. This reflects the

Table 3. HI antibodies to the five drift variants of Table 1 in pre-vaccination and 5-week post-vaccination sera of 23 HD and 26 cardiac patients and in acute and convalescent phase sera of 26 young military conscripts (MC) with a serologically confirmed influenza A infection

<table>
<thead>
<tr>
<th></th>
<th>FN/381/95</th>
<th>FN/539/97</th>
<th>FN/579/98</th>
<th>FN/680/99</th>
<th>FN/749/00</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD pre-vaccination</td>
<td>1.27 (0.55)</td>
<td>1.21 (0.52)</td>
<td>1.08 (0.24)</td>
<td>1.14 (0.15)</td>
<td>0.90 (0.30)</td>
</tr>
<tr>
<td>Protection rate (%)</td>
<td>35</td>
<td>30</td>
<td>4</td>
<td>1.14 (0.15)</td>
<td>0.90 (0.30)</td>
</tr>
<tr>
<td>HD post-vaccination</td>
<td>1.77 (0.45)</td>
<td>1.69 (0.54)</td>
<td>1.27 (0.25)</td>
<td>1.24 (0.20)</td>
<td>1.20 (0.53)</td>
</tr>
<tr>
<td>Protection rate (%)</td>
<td>78</td>
<td>65</td>
<td>26</td>
<td>4</td>
<td>26</td>
</tr>
<tr>
<td>Cardiac pre-vaccination</td>
<td>0.85 (0.32)</td>
<td>0.79 (0.24)</td>
<td>0.80 (0.19)</td>
<td>0.90 (0.15)</td>
<td>0.75 (0.16)</td>
</tr>
<tr>
<td>Cardiac post-vaccination</td>
<td>1.88 (0.77)</td>
<td>1.77 (0.75)</td>
<td>1.35 (0.37)</td>
<td>1.02 (0.22)$^a$</td>
<td>1.42 (0.53)</td>
</tr>
<tr>
<td>Protection rate (%)</td>
<td>73</td>
<td>62</td>
<td>35</td>
<td>4</td>
<td>38</td>
</tr>
<tr>
<td>MC acute</td>
<td>0.86 (0.34)</td>
<td>0.82 (0.30)</td>
<td>0.84 (0.27)</td>
<td>0.93 (0.15)</td>
<td>0.72 (0.08)</td>
</tr>
<tr>
<td>MC convalescent</td>
<td>1.73 (0.65)</td>
<td>1.64 (0.66)</td>
<td>1.43 (0.43)</td>
<td>1.17 (0.26)</td>
<td>1.14 (0.49)</td>
</tr>
<tr>
<td>Protection rate (%)</td>
<td>77</td>
<td>65</td>
<td>46</td>
<td>12</td>
<td>27</td>
</tr>
</tbody>
</table>

Mean logarithmic titres (SD) and the protection rates (percentages of subjects exhibiting post-vaccination and convalescent phase protective antibody titres, ≥ 1.60) are shown. Additionally, the pre-vaccination protection rates of HD patients are shown. The statistical significances have been calculated comparing HD patients’ post-vaccination titres to those of cardiac patients and to the convalescent titres of military conscripts.

$^aP = 0.003$. 

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higher pre-vaccination titres in the HD patients than in the other groups. Almost 90% of the HD patients had been vaccinated previously against influenza, but the cardiac patients and the conscripts were practically influenza vaccine naïve, explaining the difference in pre-vaccination titres. However, HD patients did not differ from the other two groups in the post-vaccination antibody levels and correspondingly in the proportion of the persons mounting a protective post-vaccination titre to the five different epidemic virus variants. As the vaccination of HD patients induced a significant increase in antibody levels (when compared with pre-vaccination antibody levels) against the studied virus variants except that of year 1998–1999 (FN680/99), it seems justified to propose that this particular vaccination was effective. This suggests that in the case of influenza vaccination B cells get appropriate co-stimulatory signals from antigen presenting cells and T cells, although there is a shift towards Th-1 type cell functions not favouring antibody formation in uraemia [9].

The cross-reactivity declined among all the groups until the last studied epidemic variant, FN/749/00 isolated in 2000. Surprisingly, the protection rate among all the groups increased from 4–12 (as determined against FN/680/99 isolated in 1999) to 26–38% (against FN/749/00 isolated in 2000). Our sequence analysis of viral haemagglutinin suggests that two reverted amino acid changes in the antigenic sites A (residue 144) and B (residue 160) may be involved in the enhanced cross-reactivity. Both are located in the protruding loops close to the receptor-binding site and are thus suitable for avid antibody binding and may participate in antibody-mediated neutralization and inhibition of haemagglutination [16]. Our results on the enhanced cross-reactivity suggest that the advantage of vaccination-induced immunity is not necessarily restricted only to the season following vaccination. At least occasionally, the antigenic evolution of influenza viruses may result in a situation in which previous vaccinations and natural infections have favourably primed a part of the host population. Previous studies have shown that the influence of anamnestic antigenic experiences for the vaccination-induced immunity may greatly vary from year to year depending, for example on the antigenic evolution of the influenza virus [17]. Our results are valid for H3N2 type influenza viruses, and the situation might be different with H1N1 type or with influenza B virus [5,18].

In conclusion, we found first that influenza vaccination of HD patients provokes corresponding levels of cross-reacting H3N2 subtype influenza A virus HI antibodies to cardiac patients with normal kidney function. The induced antibodies are even comparable with those detected after natural influenza A infection in young healthy males. Secondly the antigenic drift of influenza A virus may after a decline in cross-reactivity result in enhanced cross-reactivity again. This emphasizes the importance of yearly influenza vaccination, which may be advantageous not only for the corresponding season, but even for the subsequent years. Additionally, the decline of antibody levels among HD patients seen after hepatitis B vaccination [19] further underscores the need for yearly influenza vaccination, while no evidence of decreasing protection with repeated influenza vaccination has been shown [20].

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Conflict of interest statement. None declared.

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