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ANTIBODY RESPONSE TO INACTIVATED SUBUNIT INFLUENZA VACCINE IN PATIENTS WITH WEGENER'S GRANULOMATOSIS

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In the present study we investigated the humoral response to inactivated subunit influenza vaccine in patients with Wegener's granulomatosis, who were in clinical and serological remission after immunosuppressive treatment (Group I). The results were compared with patients with Wegener's granulomatosis who were treated immunosuppressively, but were not vaccinated (Group II) and with healthy persons who received the vaccine (Group III). After vaccination, antihemagglutinin and antineuraminidase antibody titers significantly increased in Groups I and Group III subjects when compared with the pre-vaccination values. Post-vaccination protection rates ranged from 51.4% to 74.3% in Group I patients and from 65.7% to 94.3% in Group III subjects. In Group II, the protection rates were between 0% and 21.4%. The response rates ranged from 60% to 74.3% in Group I patients and from 71.4% to 88.6% in Group III subjects. In Group II, the response rates were between 7.1% and 21.4%. The study confirmed the immunogenicity of influenza vaccine in patients with Wegener's granulomatosis and showed similar response in the patients to those present in healthy people.

K e y   w o r d s : *influenza, vaccine, vaccination, Wegener's granulomatosis*

INTRODUCTION

Wegener's granulomatosis is a systemic disease characterized by necrotizing granulomatous inflammation of the upper and lower respiratory tract in combination with vasculitis and focal necrotizing crescentic glomerulonephritis. The pathogenesis of the disease in not well known. A lot of medical data have

risen doubts regarding a special role of infectious agent in the induction of diseases activity. Treatment with cyclophosphamide in combination with corticosteroids has proved highly successful, although side effects may be severe and sometimes lethal. After remission is achieved, the course of a disease is highly variable and unpredictable. Most patients have relapses at variable intervals requiring reinstitution of immunosuppressive therapy.

Influenza virus remains a crucial cause of diseases and lethalness in the world. One gets infected by inhaling aerosolized particles or in the course of a direct contact. Infections caused by the virus may be very serious and can result not only in high morbidity, but also in increased mortality, mainly due to severe complications, including primary influenza pneumonia or secondary bacterial pneumonia. Most complications occur among immunosuppressed patients. Patients with Wegener's granulomatosis also account for this high risk group. The only existing and cost-effective way of protection against infections caused by this virus is seasonal vaccination, recommended by the World Health Organization and the Advisory Committee on Immunization Practices for high-risk groups (1). In spite of these clear recommendations, a small percentage of patients decide to be vaccinated. Due to insufficient knowledge or misconceptions about the benefits of immunization, many patients, and also physicians are convinced that this form of prevention is ineffective. However, there are publications showing that influenza vaccination in high risk patients is safe and also effective as in healthy people because it prevents infections with the influenza virus, or at least decreases the severity of the disease.

Patients receiving immunosuppressive and cytotoxic drugs (prednisolone, cyclophosphamide) also belong to high-risk groups in which particularly serious complications and significant numbers of death cases are registered. Chemotherapy causes immunosupresion of both cellular and humoral immunity. It is known that disturbances of the humoral response result in impaired seroconversion after vaccination. Accordingly, the aim of the present study was to assess the serological immune response to influenza vaccine in patients with Wegener's granulomatosis (ANCA-positive primary systemic vasculitis) undergoing chemotherapy. We assessed the humoral response to the inactivated subunit influenza vaccine in patients with Wegener's granulomatosis who were in clinical and serological remission after immunosuppressive treatment

MATERIAL AND METHODS

The study was performed in accordance with the Declaration of Helsinki for Human Research and was approved by a local Ethics Committee. Informed consent was obtained from all patients examined.

Thirty five patients aged 20-63 years (mean 40.5 years; median 46 years) with primary systemic vasculitis –Wegener's granulomatosis, who were in clinical and serological remission after

immunosuppressive treatment, were enrolled into the study (Group I). The inclusive criteria for this group were the following:

- ○ recognized Wegener's granulomatosis,
- ○ age between 20 and 70 years,
- ○ executed immunosuppressive treatment,
- ○ no disease activity in clinical aspect,
- ○ no disease activity in biochemical aspect,
- ○ no disease activity in serological aspect.

The results were compared with 28 patients aged 17-42 years (mean 44.7 years; median 47 years) with Wegener's granulomatosis with immunosuppressive treatment, who were not vaccinated (Group II). The inclusive criteria for this group were as follows:

- ○ recognized Wegener's granulomatosis,
- ○ age between 20 and 70 years,
- ○ currently applied immunosuppressive treatment,
- ○ disease activity in clinical aspect,
- ○ disease activity in biochemical aspect,
- ○ disease activity in serological aspect.

The third study group consisted of 35 healthy persons aged 23-79 years (mean 35.1 years; median 28 years), who received the vaccine (Group III). The inclusive criteria for this control group were the following:

- ○ no Wegener's granulomatosis recognized,
- ○ healthy volunteer,
- ○ age between 20 and 70 years,
- ○ the results of clinical, biochemical and serological examinations within norm.

Patients from Group I and healthy subjects from Group III were vaccinated against influenza before the epidemic season 2006/2007 with inactivated subunit influenza vaccine (*Influvac*, Solvay Pharmaceuticals) containing in one dose 15 µg of hemagglutinin of each of the following influenza strains recommended as the components of the influenza vaccine by WHO: A/New Caledonia/20/99 (H1N1)-like virus, A/Wisconsin/67/2005 (H3N2)-like virus or A/Hiroshima/52/2005 (H3N2) and B/Malaysia/2506/2004-like virus or B/Ohio/1/2005 (2).

The antibody response to hemagglutinin components and neuraminidase components of the influenza vaccine was assessed in serum samples obtained from blood specimens that were collected before administration of the vaccine and one month after this intervention. Antihemagglutinin (anti-HA) antibody levels and antineuraminidase (anti-NA) antibody levels were measured by hemagglutination inhibition test and neuraminidase inhibition test, respectively, at the National Influenza Center, NIH, Warsaw. Hemagglutination inhibition test was performed according to the routine technique recommended by WHO with using 0.5% turkey red blood cells (3-5). Neuraminidase inhibition test wad carried out according to Aymard-Henry`s method modified by Aymard-Henry et al (6) and Douglas (7). All sera were frozen at-20°C until use and were then incubated in a 56°C water bath for 30 min and treated with Receptor Destroying Enzyme (RDE) from *Vibrio cholerae* to remove non-specific inhibitors of hemagglutination that may be present in sera and cause false positive results in the hemagglutination inhibition test.

Based on the results obtained in both assays the following serological parameters were determined:

- ○ geometric mean titer (GMT) of anti-HA and anti-NA antibodies before and after vaccination,
- ○ mean fold increase (MFI) of anti-HA and anti-NA antibody levels after vaccination,
- ○ protection rate, i.e., the proportion of subjects with anti-HA antibody titers ≥1:40 before and after vaccination,

○ response rate, i.e., the proportion of subjects with at least a fourfold increase of anti-HA antibody titers after vaccination.

Statistical analysis was performed using a non-parametric Wilcoxon paired test and a Mann-Whitney unpaired test in Statistica software (StatSoft, 2001, version 6.0, USA).

RESULTS

Before administration of the influenza vaccine, the anti-HA antibody titers did not differ significantly (P>0.05) among the three study groups. One month after vaccination, the anti-HA antibody levels significantly increased (P<0.05) in patients with Wegener's granulomatosis who received influenza vaccine and in the healthy subjects who also received the vaccine. MFI ranged in these two groups (Group I and Group III) from 5.4 to almost 13 and from 6.7 to 24.1, respectively. In patients with Wegener's granulomatosis who did not receive the vaccine MFI the values were low and ranged from 0.9 to 1.4. The detailed results are presented in *Table 1*.

There were no significant differences in the anti-HA antibody titers between patients with Wegener's granulomatosis who were vaccinated against influenza and healthy persons with the exception of anti-HB antibodies. Their post-vaccination levels were significantly higher (P<0.05) in the healthy subjects from Group III than in the patients from Group I.

Protection rates, i.e., the percentage of subjects with the protective anti-HA antibody titers amounting to ≥1:40, did not exceed 15% in all three study groups before administration of the vaccine. One month after vaccination, the values of this parameter were significantly higher and ranged from 51.4% to 74.3% in the patients from Group I and from 65.7% to 94.3% in the healthy persons from Group III (*Table 2*). In Group I, 65.7% of patients had protective anti-HA

Table 1. Antibody response to hemagglutinin components of influenza vaccine in patients with Wegener's granulomatosis.

Group	Antigen	GMT[1] of anti-HA[2] antibodies		MFI[3] of anti-HA[2] antibody titers after administration of the vaccine
		Before	After	
		administration of the vaccine		
I – vaccinated patients	A/H1N1	5.9	75.4	12.8
II – non-vaccinated patients		7.6	10.5	1.4
III – healthy vaccinated subjects		5.9	142.1	24.1
I – vaccinated patients	A/H3N2	6.7	36.2	5.4
II – non-vaccinated patients		6.6	5.9	0.9
III – healthy vaccinated subjects		7.7	51.7	6.7
I – vaccinated patients	B	6.0	40.8	6.8
II – non-vaccinated patients		5.1	6.2	1.2
III – healthy vaccinated subjects		8.2	109.8	13.4

[1]Geometric mean titer; [2]Antihemagglutinin; [3]Mean fold increase.

Table 2. Protection rates and response rates to influenza antigens in patients with Wegener's granulomatosis.

Group	Antigen	Protection rate[1] (%)		Response rates[2] (%) after administration of the vaccine
		Before	After	
		administration of the vaccine		
I – vaccinated patients	A/H1N1	5.7	74.3	74.3
II – non-vaccinated patients		7.1	21.4	21.4
III – healthy vaccinated subjects		2.9	88.6	88.6
I – vaccinated patients	A/H3N2	5.7	51.4	60.0
II – non-vaccinated patients		3.6	3.6	10.7
III – healthy vaccinated subjects		14.3	65.7	71.4
I – vaccinated patients	B	0.0	54.3	71.4
II – non-vaccinated patients		0.0	3.6	7.1
III – healthy vaccinated subjects		11.4	94.3	85.7

[1]Percentage of subjects with the protective anti-HA antibody titers ≥1:40;
[2]Percentage of subjects with at least a 4-fold increase of anti-HA antibody titers.

antibody titers against at least two influenza vaccine strains. No protective antibody titers were found in 20% of patients with Wegener's granulomatosis. The healthy persons in Group III had protective anti-HA antibody titers against one influenza strain (11.4% of persons) or against at least two influenza strains (88.6%). In the case of patients with Wegener's granulomatosis who were not vaccinated against influenza, the protection rates were low and ranged from 3.6% to 21.4% (*Table 2*).

Response rates, i.e., the percentage of subjects with at least a 4-fold increase of anti-HA antibody titers after administration of the vaccine, ranged from 60.0% to 74.3% in the patients from Group I and from 71.4% to 88.6% in the healthy persons from Group III (*Table 2*). In patients with Wegener's granulomatosis who did not received the vaccine, the response rates ranged from 7.1% to 21.4% (*Table 2*).

Before administration of the influenza vaccine, similarly to anti-HA antibodies, the titers of anti-NA antibodies did not differ significantly (P>0.05) among the three study groups. A single exception found concerned the case of anti-NB antibodies, as their significantly higher levels were registered in the patients from Group I compared with those in the healthy persons from Group III. One month after vaccination, the anti-NA antibody levels significantly increased in the patients with Wegener's granulomatosis who received influenza vaccine and in the healthy subjects. MFI of anti-NA antibody titers ranged from 3.0 to 8.4 (Group I) and from 5.4 to 10.2 (Group III) (*Table 3*). Significantly higher post-vaccination anti-N1 titers were found in Group I than in Group III, but, on the other hand, significantly higher post-vaccination anti-NB titers were found in Group III than in Group I. In the case of patients who were not vaccinated, the MFI values were between 0.9 and 1.1, and the anti-NA antibody titers did not differ significantly (P>0.05) through the whole study period (*Table 3*). In Group II consisted of non-vaccinated patients with Wegener's granulomatosis, the anti-

Table 3. Antibody response to neuraminidase components of influenza vaccine in patients with Wegener's granulomatosis.

Group	Antigen	GMT[1] of anti-NA[2] antibodies		MFI[3] of anti-NA[2] antibody titers after administration of the vaccine
		Before	After	
		administration of the vaccine		
I – vaccinated patients	A/H1N1	6.3	52.8	8.4
II – non-vaccinated patients		6.4	5.8	0.9
III – healthy vaccinated subjects		7.0	39.2	5.6
I – vaccinated patients	A/H3N2	7.1	39.2	5.5
II – non-vaccinated patients		6.4	7.2	1.1
III – healthy vaccinated subjects		7.1	38.4	5.4
I – vaccinated patients	B	6.7	20.4	3.0
II – non-vaccinated patients		6.6	5.8	0.9
III – healthy vaccinated subjects		5.2	52.8	10.2

[1]Geometric mean titer; [2]Antineuraminidase; [3]Mean fold increase.

HA and anti-NA antibody levels were significantly lower ($P<0.05$) than those in Group I and Group III.

No serious adverse reactions or cases of deterioration were registered after administration of the vaccine.

DISCUSSION

Up to now, only few sources concerning the estimation of vaccination effectiveness in patients with connective tissue diseases (lupus) have been published (8-10). However, any medical data on patients with Wegener's granulomatosis and influenza vaccinations, or vaccinations in general, are missing. Moreover, it is commonly known that virus infections trigger the development of bacterial infections, especially those caused by *Staphylococcus aureus* (11). Recently, it has been noted that patients with Wegener's granulomatosis frequently have symptoms of respiratory tract infection. Previous studies have also found that many patients have respiratory tract infection either at onset of the disease or before a relapse. Virtually every patient with Wegener's granulomatosis has secondary infections of the paranasal tissues, predominantly with *Staphylococcus aureus*, and infection invariably respond to antistaphilococcal antibiotics (12, 13). Nasal carriage of *Staphylococcus aureus* is considered a risk factor for *Staphylococcus aureus* infections and is responsible for initiating a disease *de novo* or its relapse. It is very hard, even for the experienced physician, to tell a difference between the diseases relapse and a common virus infection, including influenza virus infection, in Wegener's granulomatosis patients. It is one of the main, unexplained problems in diagnosing and treatment of those patients. In patients with Wegener's granulomatosis antineutrophil cytoplasmic antibodies (ANCA) directed against

either proteinase 3 (PR3) or myeloperoxidase (MPO) may still be elevated despite the clinical remission (14). Hence, these antibodies should be analyzed carefully at relapse suspicion. Misinterpretation of the patients' clinical condition, i.e., relapse recognition in patient with influenza or other viral infection, exposes the patient to a risk of side effects in the course of therapy, including death.

Thus, the above-mentioned important arguments should be taken into consideration both by patient and physician before the vaccination against influenza will be rejected. Another reason supporting a need of influenza vaccinations in patients with Wegener's granulomatosis, and in other groups with medical problems as well, is a high cost of treatment of these patients. Therefore, infection with influenza virus in patients with Wegener's granulomatosis, on the one hand, may cause deterioration and, on the other hand, may seriously increase economic and social costs resulted from influenza illness and possible post-influenza complications (15-17).

Due to the lack of data regarding immunological response to influenza vaccination in patients with Wegener's granulomatosis, the results of the present study may fill in this gap and provide information of practical significance. It should be emphasized that despite clear ACIP recommendations and opinions of various scientific societies recommending influenza vaccinations for specific groups, many patients do not use this form of prophylaxis against influenza, and some physicians do not offer influenza vaccinations to their patients due to insufficient knowledge about the efficacy and safety of influenza vaccines, even if inactivated influenza vaccines containing disrupted virions or only surface glycoproteins are taken into consideration (1). Previous studies performed by the National Influenza Center, NIH, in Warsaw, Poland together with clinicians engaged with different high-risk groups of patients showed that inactivated split or subunit influenza vaccines are safe and immunogenic, and antibody response is many a time comparable with that in healthy people (18-23).

The protective effect of influenza vaccine is connected with anti-HA antibodies. The results of this study showed that the antibody response to two (H1 and H3) of the three hemagglutinin components of influenza vaccine in patients with Wegener's granulomatosis was comparable with that in healthy subjects. Moreover, MFI values of anti-HA antibody titers and response rates for all three influenza antigens (A/H1N1, A/H3N2, B) were higher than 2.5 and 40%, respectively. This means that the requirements of the Committee for Proprietary Medicinal Products (CPMP) established for the antibody response to influenza vaccination were fulfilled, and it should be noticed that the above requirements regard healthy adults, and not persons with medical disorders such as Wegener's granulomatosis (23). Values of the other parameter of the humoral response to influenza vaccination, i.e., the protection rate also fulfilled the requirements of CPMP, but only in the case of the response to antigen A/H1N1. According to CPMP, protection rate should amount to at least 70% in healthy adults and at least 60% in people over 60 years (24). In the case of response to influenza antigen B, 54.3% of patients had anti-HA

antibodies in the protective titers in comparison with 94.3% of healthy persons. Similarly, the protection rate for antigen A/H3N2 was lower than the required 70% or 60%, because it amounted to 51.4%. Nevertheless, a low protection rate (65.7%) was also registered for this antigen in healthy persons from Group III.

Anti-NA antibodies do not prevent influenza infection, but inhibit the release of mature viral particles from infected cells which results in limitation of viral spread. Humoral response to neuraminidase component of influenza vaccine is assessed very seldom due to complicated technique of measurement and due to the fact that the quantity of neuraminidase glycoprotein in individual dose of influenza vaccine is not standardized and there are no estimated levels of anti-NA antibodies that could be considered to inhibit replication of influenza virus. The above reasons make the interpretation of the results of anti-NA antibody response very difficult. Despite these problems, anti-NA antibody levels were measured in this study and the results showed that influenza vaccine induces also the production of antibodies to neuraminidase glycoprotein and they contribute, although not on the first line, to the defense against influenza illness.

As it was already mentioned, there is lack of data regarding immunological response to influenza vaccination in patients with Wegener's granulomatosis. For this reason, a comparison of the results of this study with findings of other authors was not possible. Nevertheless, the statistical analysis of the obtained results and reference to the requirements of CPMP allow to make a conclusion that the inactivated subunit influenza vaccine is highly immunogenic and safe in patients with Wegener's granulomatosis. The protection rates lower than required by CPMP that were registered in the case of the response to antigens A/H3N2 and B should not be a reason for rejection of influenza vaccination in this group. It should be emphasized that in this kind of study an entire study group and each individual patient should be taken into consideration. Influenza vaccine ensured the production of anti-HA antibodies in the protective tiers in the majority of patients, because almost 66% of them had protective anti-HA antibody titers against at least two influenza vaccine strains. In some patients who were not able to achieve sufficiently high anti-HA antibody levels, administration of this vaccine did not present any risk as this vaccine contained only surface influenza glycoproteins. Thus, there is no better choice than to offer seasonal influenza vaccinations with inactivated influenza vaccines for patients from high-risk groups, including those with Wegener's granulomatosis. The results of this study should convince physicians to use this form of prophylaxis in their patients.

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