

Original Article

# Attenuated Antibody Reaction for the Primary Antigen but not for the Recall Antigen of Influenza Vaccination in Patients with Non-Hodgkin B-Cell Lymphoma after the Administration of Rituximab-CHOP

Tohru Takata,<sup>1)</sup> Junji Suzumiya,<sup>1,2)</sup> Takahiko Ishikawa,<sup>1)</sup> Yasushi Takamatsu,<sup>1)</sup> Hideyuki Ikematsu,<sup>3)</sup>  
and Kazuo Tamura<sup>1)</sup>

To assess the humoral response to the influenza vaccine in patients undergoing R-CHOP therapy (rituximab combined with cyclophosphamide, doxorubicin, vincristine, and prednisolone) for non-Hodgkin lymphoma (NHL), the anti-hemagglutinin (HA) titer in 7 NHL patients undergoing therapy was compared with those in 10 control group subjects in the 2005/2006 season. Four weeks after vaccination, the HA titers against the influenza type A H1N1 and type B antigens, the same antigens that had been used in the previous seasons, were elevated in all patients treated with R-CHOP. In contrast, there was no increase in the geometric mean titer for type A H3N2 antigen, which was newly included in 2005/2006 season, in the patients treated with R-CHOP, while there was a significant increase in the 10 control subjects ( $p = 0.014$ ). This study showed that vaccination against influenza virus generated an appreciable humoral response to recall antigens in NHL patients treated with R-CHOP therapy, but not to the primary antigen. [*J Clin Exp Hematopathol* 49(1) : 9-13, 2009]

**Keywords:** R-CHOP, non-Hodgkin lymphoma, influenza vaccination, primary antigen, recall antigen

## INTRODUCTION

Influenza is one of the most common infectious diseases, affecting people of all age groups and influencing morbidity and mortality worldwide. To date, the most effective method of protection against infection with the influenza virus is influenza vaccine, and annual vaccination for immunocompromised patients is recommended.<sup>1</sup> Patients with non-Hodgkin lymphoma (NHL) are at special risk of influenza virus infection because of a constitutive immunodeficiency, intensified by immunotherapy, chemotherapy, or radiotherapy and usually old age, which may also adversely affect the

immunological response to viral infections, thereby inhibiting the immune response to vaccines. However, the data on the immunogenicity of influenza vaccination in patients with NHL tend to vary greatly. In some studies, the influenza vaccine has been reported to be less immunogenic in NHL patients in comparison to healthy people, despite appropriate vaccination,<sup>2-5</sup> while in another study the influenza vaccination was reported to induce a sufficient immune response in patients with NHL, irrespective of any previous chemotherapy.<sup>6</sup>

Rituximab, a chimeric monoclonal antibody directed against the cell surface antigen CD20 of B cells, has been demonstrated to be an effective treatment for non-Hodgkin B-cell lymphoma, in which CD20 is expressed at the surface of malignant cells, and treatment with rituximab combined with CHOP (cyclophosphamide, adriamycin, vincristine, and prednisolone) (R-CHOP), is now recognized as the standard therapy for CD20-positive aggressive B-cell lymphoma.<sup>7,8</sup> Although rituximab induces an almost complete depletion of normal B lymphocytes in the peripheral blood for an average of 6-9 months, it is unusual for treatment by rituximab alone to result in suppression of serum immunoglobulin or infective complication.<sup>9</sup> However, data on the effect of R-CHOP therapy on the immune response to active immunization with influenza vaccine in NHL patients are scarce. Therefore, we

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Received : August 1, 2008

Revised : December 16, 2008

Accepted : December 24, 2008

<sup>1)</sup>Division of Oncology, Hematology, and Infectious Diseases, Department of Medicine, Fukuoka University School of Medicine, Fukuoka, Japan

<sup>2)</sup>Second Department of Internal Medicine, Chikushi Hospital, Fukuoka University, Fukuoka, Japan

<sup>3)</sup>Department of Clinical Research, Hara-Doi Hospital, Fukuoka, Japan

Address correspondence and reprint request to Tohru Takata, M.D., Ph.D., Division of Oncology, Hematology, and Infectious Diseases, Department of Medicine, Fukuoka University School of Medicine, Nanakuma 7-45-1, Jonan-Ku, Fukuoka 814-0180, Japan

E-mail : takattol@cis.fukuoka-u.ac.jp

conducted a preliminary investigation to evaluate the effect of R-CHOP therapy on the immunogenicity of vaccination against influenza and to assess vaccination safety in NHL patients.

## PATIENTS AND METHODS

### *Characteristics of patients and control subjects*

The patient characteristics are shown in Table 1. Seven patients with NHL (age, 39-71 years; median, 49 years; 3 with diffuse large B-cell lymphoma, 3 with follicular lymphoma, and 1 with small lymphocytic lymphoma) were treated with 6 cycles of CHOP therapy combined with rituximab (R-CHOP) (375 mg/m<sup>2</sup> intravenously, 6 doses, every 3 weeks). Three patients were vaccinated during the 6 cycles of R-CHOP therapy, 3 within 2 months after the 6th cycle, and one 11 months after the R-CHOP therapy. Two patients with NHL (62 and 64 years of age, respectively), treated with only 6 cycles of CHOP therapy (one was in the course of the CHOP therapy and the other was 1 year after the CHOP therapy) without rituximab (CHOP alone), were compared as a control. Rituximab was not used for the latter patient because rituximab was not yet available at that time. To confirm the immunogenicity of the vaccine, 8 healthy subjects were also included as healthy controls (age, 31-45 years; median, 39 years). The vaccinations consisted of 0.5 mL split virion inactivated vaccine for 2005-2006 (Hokken, Saitama, Japan) containing a 15 mg hemagglutinin (HA) dose of A/New Caledonian/20/99 (NC, H1N1), A/New York/55/04 (NY, H3N2), and B/Shanghai/361/02 (SHAN), administered subcutaneously.

### *Hemagglutination inhibition test*

The immunogenicity of the vaccine was tested by the hemagglutination inhibition (HI) test. HI antibody titers were measured just before vaccination and 28 days later. The serum samples were separated and stored frozen at -20°C until tested. For the HI test, the serum samples were serially diluted from 1:10 to 1:1,280 and co-incubated with 0.5% turkey red blood cells and influenza strains. The HI titer was determined as the reciprocal of the highest serum dilution causing complete inhibition of the agglutination of red blood cells.

The humoral response to the 3 antigens contained in the influenza vaccine was assessed by calculating the following parameters: geometric mean titers (GMT), mean fold increase (MFI), the seroresponse rate (the percentage of subjects with a 4-fold or more increase), and the seroprotection rate (the percentage of subjects with an HI titer of at least 1:40) in the HI titers.

### *Outcomes of the study*

A satisfactory humoral response was defined as either a positive seroresponse or positive seroprotection. The titer of an antiserum not showing any inhibition was recorded as < ×10. A complete blood count was done for all treated patients to assess the total lymphocyte count.

Upon requesting influenza vaccination, appropriate informed consent was obtained from each of the subjects involved in this pilot study.

### *Statistical methods*

The statistical significance of the comparisons of the mean values was assessed by either the non-parametric Mann-

**Table 1.** Characteristics of patients at baseline (day 0)

Clinicohistological findings	R-CHOP (n = 7)	CHOP alone (n = 2)	Healthy control (n = 8)
Age (years)	54.4 ± 11.0	63	41.4 ± 11.7
Male, sex	4	1	8
Histology			
DLBCL	3	1	
FL	3	0	NA
SL	1	0	
PT	0	1	
Lymphocytes (/μL)	644 ± 221	1,254	NE
Serum IgG levels (mg/dL)	930 ± 359	771	1,059 ± 224

Data are shown as mean ± SD. DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; SL, small lymphocytic lymphoma; PT, peripheral T-cell lymphoma; R-CHOP, CHOP therapy with rituximab; CHOP alone, CHOP therapy without rituximab; CHOP denotes cyclophosphamide, dexamethasone, vincristine, and prednisone; NE, not examined; NA, not applicable

Whitney U test or two-tailed Fisher's exact test. Probability ( $p$ ) values  $< 0.05$  were considered to be statistically significant.

## RESULTS

At the time of vaccination, lymphocytopenia ( $< 1,000/\mu\text{L}$ ) was seen in 5 of 7 patients treated with R-CHOP (Table 1). Two patients who were within 2 months of the completion of R-CHOP therapy showed a low titer of IgG, although there was no significant difference in the IgG value between the R-CHOP group and the non-R-CHOP group, which included healthy subjects. Six patients (85.7%) treated with R-CHOP, 1 patient (50.0%) treated with CHOP alone, and 8 (100%) healthy subjects had a history of influenza vaccination in the last few years before the 2005-2006 season.

The GMT, MFI, and rates of seroprotection and seroresponse of the patients treated with R-CHOP, CHOP alone, and the healthy controls are shown in Table 2. There were no significant differences in the pre-vaccination GMT of the HI antibodies, e.g. as a result of previous infection or vaccination between the patients with the R-CHOP therapy and the non-R-CHOP group, which included healthy subjects. The two patients treated with CHOP alone, who lacked a history of vaccination, were negative for all of the antibodies before vaccination. Four weeks after vaccination, positive seroprotection was seen in 6 of 7 against the influenza type A H1N1 (NC) and in all 7 against type B (SHAN) antigens in patients treated with R-CHOP, for which the same antigens had been used in the previous seasons. There was no significant difference in the GMT among the R-CHOP group and the non-R-CHOP control group (Table 2). In contrast, there was no increase in the GMTs of the HI antibody for the type A H3N2 (NY) antigen, which was newly included in the 2005-2006 season, by the patients treated with R-CHOP, but there was a significant increase for the healthy controls ( $p = 0.029$ ) and for all 10 control subjects, including the two patients who were treated with CHOP alone ( $p = 0.014$ ), thus suggesting a satisfactory immunogenicity of the NY antigen. The MFI was significantly lower for the R-CHOP group than for the healthy control subjects ( $p = 0.014$ ) and for all control subjects ( $p = 0.0007$ ). The response of the NY antigen was significantly lower for patients treated with R-CHOP (1 of 7, 14.2%) in comparison to that of the control subjects (8 of 10, 80.0%) ( $p = 0.015$ ). Only 1 patient treated with R-CHOP showed a positive titer for the H3N2 (NY) antigen (1:40) at the time of vaccination, thus suggesting a previous exposure to the antigen. A response to more than 1 antigen was obtained by all but 1 of the R-CHOP patients, and all of the 10 control subjects. No difference was noted between the R-CHOP and the control group subjects in the response rate to all 3 antigens or to none of them. In the patients receiving R-CHOP therapy, there was no apparent association between the

humoral response and age, gender, disease duration, histology of lymphoma, or the time interval since receiving the last rituximab. In addition, vaccination against influenza was not associated with a significant worsening of clinical or laboratory indices such as fever or liver dysfunction and none of the patients receiving R-CHOP therapy developed influenza during this influenza season.

## DISCUSSION

Vaccination against influenza generated a humoral response for 2 of the 3 antigens tested (NC and SHAN but not NY) in NHL patients treated with R-CHOP therapy, suggesting that the vaccine was not uniformly immunogenic among the antigens. The immune responsiveness for the recall antigens (NC and SHAN) that had been used in the previous season was comparable to the control group, and achieved titers of functional antibodies greater than the protective threshold, irrespective of previous chemotherapy administration. In contrast, the antibody titer against the primary antigen (NY), which was a newly included antigen in the 2005-2006 season, was not elevated ( $< \times 40$ ) in any of the patients treated with R-CHOP.

The immune responsiveness of patients treated with rituximab has been addressed by several studies of patients with lymphoma. Horwitz *et al.* evaluated the ability of 35 patients with lymphoma, who were being treated with rituximab and cyclophosphamide, to respond to vaccination against tetanus, *Haemophilus influenzae*, and pneumococcus administered at 6 and 9 months after their last rituximab infusion.<sup>10</sup> Most of the patients produced protective antibody levels against hemophilus and tetanus but not against pneumococcus, and the pre-existing antibody levels against tetanus and pneumococcal polysaccharide were shown to be unaffected by a single course of rituximab. Interestingly, results similar to our observations have also been reported in other studies. van der Kolk *et al.*<sup>11</sup> reported the effect of treatment with rituximab on the humoral immune response to 2 primary antigens (keyhole limpet hemocyanin and hepatitis A vaccine) and 2 recall antigens (tetanus toxoid and poliomyelitis vaccine) in 11 patients with relapsed, low-grade lymphoma. None of the patients responded to the primary antigens before or after the treatment. In contrast, all patients responded to recall antigens, although the response was significantly lower than that before treatment.<sup>11</sup> Oren *et al.* reported that rheumatoid arthritis patients treated with rituximab had low response to a different H3N2 antigen (California) than the one used in the present study, that was included in the vaccine of the 2005-2006 season, while the response rate to the other 2 antigens was similar among all patients.<sup>12</sup> These results suggest that lower responsiveness to primary antigens may be common to rituximab treatment.

The two recent studies may explain in part why the re-

**Table 2.** Antibody kinetics in patients with R-CHOP therapy and control vaccinated against influenza in the epidemic season 2005-2006

Subjects	GMT	MFI	Protection	Response
A/New Caledonia/20/99 (H1N1)				
Pre-				
R-CHOP	36.23			
CHOP alone	14.14			
Healthy donor	80.00	NA	NA	NA
Control, total	56.57			
28 days post-				
R-CHOP	48.76	1.49	86%	29%
CHOP alone	28.28	2.00	50%	0%
Healthy donor	95.14	1.19	88%	13%
Control, total	74.67	1.32	80%	10%
A/New York/55/04 (H3N2)				
Pre-				
R-CHOP	13.46			
CHOP alone	14.14			
Healthy donor	11.89	NA	NA	NA
Control, total	12.31			
28 days post-				
R-CHOP	13.46	1.00	29%	0%
CHOP alone	56.57	4.00	50%	100%
Healthy donor	47.57 <sup>a</sup>	4.00 <sup>c</sup>	63%	75%
Control, total	49.25 <sup>b</sup>	4.00 <sup>d</sup>	60%	80% <sup>e</sup>
B/Shanghai/361/02				
Pre-				
R-CHOP	36.23			
CHOP alone	10.00			
Healthy donor	47.57	NA	NA	NA
Control, total	34.82			
28 days post-				
R-CHOP	53.84	1.81	100%	29%
CHOP alone	40.00	4.00	50%	100%
Healthy donor	61.69	1.35	100%	0%
Control, total	56.57	2.00	90%	20%

GMT ; geometric mean titer, MFI ; mean fold increase, NA ; not applicable, R-CHOP ; CHOP therapy with rituximab, CHOP alone ; CHOP therapy without rituximab. CHOP denotes cyclophosphamide, doxorubicin, vincristine, and prednisone. The differences between groups were tested in Mann-Whitney unpaired test (a-d) or Fisher's exact test (e), and  $p < 0.05$  was considered as significant.

sponse to the recall antigen is maintained to some degree after treatment with rituximab. Mamani-Matsuda *et al.* demonstrated the persistence of CD27<sup>+</sup>IgG<sup>+</sup> memory B cells in the spleen in contrast to the decrease or depletion of memory B cells in the peripheral blood after the treatment with rituximab.<sup>13</sup> Ahuja *et al.* demonstrated that the plasma cells, which pool in spleen and bone marrow, do not need a significant supply from memory B cells for their maintenance and

that they live long enough to maintain the antibody titers over a long period without renewal.<sup>14</sup> These findings suggest that depletion of memory B cells in peripheral blood with rituximab should have no effect on either the number of CD27<sup>+</sup>IgG<sup>+</sup> memory B cells or long-lived plasma cells, thus leading to the maintenance of the response to the recall antigen.

The roles of CHOP therapy and rituximab in response to the vaccination profiles remain to be elucidated. It is not

certain whether antibody production after influenza vaccination was attenuated by the effect of both CHOP and rituximab, or only by rituximab. However, the normal antibody production after vaccination of the NY antigen in the 2 NHL patients who were treated with CHOP alone suggests that rituximab was responsible for the attenuation of the response to the primary antigen.

This preliminary study demonstrates that the vaccination of B-cell lymphoma patients against influenza is safe. These findings also suggest that an appreciable humoral response to recall antigens, but not to the primary antigen, is generated in NHL patients treated with R-CHOP. Larger studies to evaluate the respective impact of rituximab and CHOP on the immunogenicity of influenza vaccination are necessary to confirm these findings.

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