Antibody Responses of Splenectomized Patients with Non-Hodgkin’s Lymphoma to Immunization with Polyvalent Pneumococcal Vaccines

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The serum antibody responses of splenectomized patients with non-Hodgkin’s lymphoma (NHL) who had been immunized with a polyvalent pneumococcal vaccine (Pneumovax 23) were evaluated by an enzyme-linked immunosorbsorbent assay with the 23-valent pneumococcal vaccine as the antigen. A response to immunization, defined as a twofold-or-higher rise of the prevaccination titer of antibodies against Streptococcus pneumoniae polysaccharide, was elicited in 5 of 11 patients with NHL. No significant difference in the level of antibodies against S. pneumoniae polysaccharide between lymphoma patients and patients who had undergone splenectomy for other reasons was detected (P = 0.83 and 0.87 before and after vaccination, respectively). NHL patients who did not respond to the first immunization received a booster dose of the polysaccharide vaccine. This injection did not increase the pneumococcal-antibody titer significantly (P = 0.7). We conclude that vaccination with pneumococcal polysaccharides in splenectomized patients with NHL elicits an adequate antibody response in 45.4% of the cases and should therefore be administered. Revaccination of the nonresponders does not further increase the pneumococcal-antibody levels.

Patients who have undergone splenectomy are at risk for infections involving Streptococcus pneumoniae, such as fulminating bacteremia (16, 38). Invasive pneumococcal infections in splenectomized patients result in a mortality rate of up to 75% (10, 16, 20) and are more likely to occur if additional risk factors are present. Thus, asplenic patients undergoing intensive chemotherapy and irradiation, e.g., for lymphoma or leukemia, are at risk for overwhelming sepsis with S. pneumoniae (37).

More than 84 serological types of this bacterium have been identified so far (19). The pneumococcal serotypes most frequently responsible for infection are types 14, 4, 23F, 9V, 3, and 6B in North America (33) and types 3, 14, 19F, 6B, 20, 4, 7F, and 10A in Germany (23). Prophylaxis with pneumococcal vaccines was introduced in 1977 (36). The vaccines are both immunogenic and protective against infections with S. pneumoniae in immunocompetent patients (4). However, immunization with pneumococcal polysaccharides has been shown to be less effective in patients with leukemia or disseminated cancer (33).

Several investigators have studied the antibody responses to pneumococcal vaccines in asplenic patients with Hodgkin’s disease (13, 25, 35), but little is known about the protective effect of vaccination before chemo- or radiotherapy, and so far no data are available on posttreatment boosters in splenectomized patients with non-Hodgkin’s lymphoma (NHL). However, patients with NHL of the B-cell type are at the highest risk for infection with S. pneumoniae due to the state of immunodeficiency associated with this malignancy (3, 21).

The aim of this study was to evaluate the response to the pneumococcal vaccine in splenectomized patients with B-cell NHL. One dose of vaccine containing capsular polysaccharides of 23 different serotypes was injected into patients prior to treatment of the lymphoma. The serum antibody response to the 23-valent vaccine was determined by enzyme-linked immunosorbent assay (ELISA). Depending on the pneumococcal antibody status after primary immunization and at the end of irradiation and chemotherapy, the patients were revaccinated.

MATERIALS AND METHODS

Patient characteristics. Eleven splenectomized, unvaccinated patients with B-cell NHL (Table 1) and seven patients who had undergone splenectomy for other reasons (Table 2) were included in the study. The median ages were 52 years for the lymphoma patients (range, 34 to 69 years) and 45 years for the patients with nonneoplastic diseases (range, 20 to 83 years). Subsequent to immunization, multiagent chemotherapy was given to five NHL patients; this treatment consisted of the intermittent administration of COP (cyclophosphamide, vincristine, prednisone), CHOP (cyclophosphamide, vincristine, doxorubicin, prednisone), Pro-MACE (cyclophosphamide, methotrexate, doxorubicin hydrochloride [Adriamycin], etoposide), or fludarabine. COP combined with involved-field irradiation (25.5 Gy) and extended-field irradiation alone (36 Gy) were administered to two further lymphoma patients (Table 1).

Antineoplastic therapy was started on the day after vaccination in one case (patient 9 [Table 1]) or after an interval of 4 weeks in the remaining patients. Five patients (patients 1, 4, 5, 8, and 11 [Table 1]) had serum gamma globulin concentrations below 10%. Four lymphoma patients (patients 1 to 4 [Table 1]) were not treated with tumor therapy subsequent to immunization. Control patients with nonneoplastic diseases were given neither steroids nor immunosuppressive treatment at the time of immunization.

Vaccination. Immunization was administered immediately prior or subsequent to splenectomy. Patients received 0.5 ml of a 23-valent pneumococcal capsular polysaccharide vaccine (Pneumovax 23; Behringwerke, Marburg, Germany). The vaccine contained 25 μg of each capsular polysaccharide (Danish types 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F). This preparation was given intramuscularly in the left deltoid. Serum samples were taken at three times: before vaccination, 5 to 15 weeks after vaccination, and 4 to 7 months after vaccination. Patients who did not respond to the primary vaccination and patients who were in remission or who had minimal disease 2 to 3 months after completion of the irradiation and/or chemotherapy received a 0.5-ml booster dose of the vaccine. All patients receiving a second immunization had normal serum gamma globulin concentrations (>10%) at the
TABLE 1. Antibody response to pretreatment immunization with pneumococcal polysaccharide vaccine in splenectomized patients with NHL

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Diagnosis</th>
<th>Post-vaccination treatment</th>
<th>Titer of antibodies against Pneumovax 23</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before immunization</td>
<td>After immunization</td>
</tr>
<tr>
<td>1</td>
<td>MALT</td>
<td>CTX RX</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>Immunocytoma</td>
<td>– –</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>ML, unclassified</td>
<td>– –</td>
<td>195</td>
</tr>
<tr>
<td>4</td>
<td>CB-CC</td>
<td>– –</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>Immunocytoma</td>
<td>+ –</td>
<td>&lt;25</td>
</tr>
<tr>
<td>6</td>
<td>Immunocytoma</td>
<td>+ –</td>
<td>125</td>
</tr>
<tr>
<td>7</td>
<td>MALT</td>
<td>+ +</td>
<td>31</td>
</tr>
<tr>
<td>8</td>
<td>CB-CC</td>
<td>– +</td>
<td>39</td>
</tr>
<tr>
<td>9</td>
<td>ML, B-cell type</td>
<td>+ –</td>
<td>189</td>
</tr>
<tr>
<td>10</td>
<td>Immunocytoma</td>
<td>+ –</td>
<td>124</td>
</tr>
<tr>
<td>11</td>
<td>CB</td>
<td>+ –</td>
<td>110</td>
</tr>
</tbody>
</table>

* Splenectomized patients were immunized with a 23-valent pneumococcal polysaccharide vaccine. Antibody response to the vaccine was determined by ELISA.

** MALT, mucosa-associated lymphatic tissue lymphoma; ML, malignant lymphoma; CB-CC, centroblastic-centrocytic lymphoma; CB, centroblastic lymphoma.

a CTX, multiagent chemotherapy; RX, irradiation therapy.

time of the booster immunization. Serum samples were obtained before reimmunization and 5 to 15 weeks after reimmunization.

Antibody assay. The levels of immunoglobulin G antibodies to Pneumovax 23 in serum were determined by an ELISA with Pneumovax 23 as the antigen (14). Because of the poor binding of carbohydrates to plastic surfaces, the carbohydrate mixture was derivatized according to the method of Barrett et al. (6). In brief, the content of each of five vials of Pneumovax 23 (0.5 ml containing 23 25-μg portions of capsular polysaccharide corresponding to the 23 pneumococcal types) was added to 1.5 ml of 0.01 M NaOH and mixed immediately. The solution was transferred to a tube containing 6.5 mg of cyanuric chloride (Sigma Chemical Co., St. Louis, Mo.) and mixed gently with continuous checking of the pH. After 5 min at pH 8.3, the solution was transferred to a tube containing a solution of 0.3 mg of poly-L-lysine in 0.3 ml of 0.05 M Tris buffer (pH 8.1). The solution was dialyzed against phosphate-buffered saline (PBS), pH 7.4. It was then diluted to 100 ml with PBS (pH 7.4), dispensed in 100-μl samples onto microtiter plates (Maxisorp; Nunc, Roskilde, Denmark) and incubated for 24 h at room temperature. The plates were washed three times with distilled water and incubated for 10 min with washing buffer (PBS, pH 7.4, containing 0.05% Tween 20 and 0.5% NaCl) to block the surface hydrophobic capacity. The PSG was used for coating of the microtiter plates.

The mean antibody titers after vaccination with S. pneumoniae were encountered in a few cases. Two patients with idiopathic thrombocytic purpura had local redness and induration at the site of injection for 2 to 3 days. One of these patients had a fever of 39°C for 1 day; the other patient complained of dizziness and chills.

Antibody response to initial immunization. Serum Anti-Pv titers after vaccination with S. pneumoniae polysaccharides in patients with NHL are listed in Table 1. For one of four NHL patients not treated with cytoreductive agents subsequent to immunization (patient 3 [Table 1]), and for two of seven patients treated with irradiation and chemotherapy (patients 8 and 9, respectively [Table 1]), a significant rise in the prevaccination titer was detected in the first serum sample (5 to 15 weeks after immunization). In the second serum sample (4 to 7 months after immunization) for 4 of 11 patients (patients 2, 3, 5, and 8), a significant increase in anti-Pv titers was detected (total response rate, 5 of 11 patients [45.4%]). In 51.8% of the splenectomized patients with Neoplastic diseases, a significant rise in the anti-Pv titer was measured subsequent to immunization. There was no statistically significant difference (P = 0.87) in anti-Pv titers between the patients with NHL (mean anti-Pv titer = 1:742) and the controls (mean anti-Pv titers = 1:866) at the end of the observation period.

Antibody response to booster immunization. Four patients with NHL and a low anti-Pv titer after primary immunization were revaccinated with 0.5 ml of Pneumovax 23. Two patients developed local erythema at the site of injection, while no adverse reactions were observed in the other two patients.

Antibody response to booster immunization. The mean anti-Pv titer before revaccination (1:159) did not differ significantly from the pretreatment value (1:125). The antibody titers were measured after 1 month (mean anti-Pv titer = 1:496) and 6 months (mean anti-Pv titer = 1:604).

TABLE 2. Antibody response to immunization with pneumococcal polysaccharide vaccine in splenectomized patients with nonneoplastic diseases

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Titer of antibodies against Pneumovax 23</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before immunization</td>
</tr>
<tr>
<td></td>
<td>5–15 wk</td>
</tr>
<tr>
<td>Hemolytic anemia</td>
<td>200</td>
</tr>
<tr>
<td>ITP</td>
<td>367</td>
</tr>
<tr>
<td>ITP</td>
<td>&lt;25</td>
</tr>
<tr>
<td>Biliary cirrhosis</td>
<td>&lt;25</td>
</tr>
<tr>
<td>ITP</td>
<td>318</td>
</tr>
<tr>
<td>Hemolytic anemia</td>
<td>25</td>
</tr>
<tr>
<td>ITP</td>
<td>119</td>
</tr>
</tbody>
</table>

* Splenectomized patients were immunized with a 23-valent pneumococcal polysaccharide vaccine. Patients had been given neither steroids or immunosuppressive treatment at the time of immunization. ITP, idiopathic thrombocytopenic purpura; ND, not done.

RESULTS

Antibody titers before immunization. The titers of antibodies against S. pneumoniae before vaccination are listed in Table 1 for the 11 NHL patients and in Table 2 for the 7 patients with nonneoplastic diseases. The titers of natural antibodies against the antigens of the 23-valent polysaccharide vaccine (anti-Pv) were lower in the lymphoma patients than in the controls (the mean titers were 1:88 and 1:154, respectively), but the difference was statistically not significant (P = 0.83).

Adverse reactions. Mild adverse reactions to immunization with the 23-valent pneumococcal vaccine were encountered in a few cases. Two patients with idiopathic thrombocytic purpura had local redness and induration at the site of injection for 2 to 3 days. One of these patients had a fever of 39°C for 1 day; the other patient complained of dizziness and chills.

Antibody response to initial immunization. Serum Anti-Pv titers after vaccination with S. pneumoniae polysaccharides in patients with NHL are listed in Table 1. For one of four NHL patients not treated with cytoreductive agents subsequent to immunization (patient 3 [Table 1]), and for two of seven patients treated with irradiation and chemotherapy (patients 8 and 9, respectively [Table 1]), a significant rise in the prevaccination titer was detected in the first serum sample (5 to 15 weeks after immunization). In the second serum sample (4 to 7 months after immunization) for 4 of 11 patients (patients 2, 3, 5, and 8), a significant increase in anti-Pv titers was detected (total response rate, 5 of 11 patients [45.4%]). In 51.8% of the splenectomized patients with nonneoplastic diseases, a significant rise in the anti-Pv titer was measured subsequent to immunization. There was no statistically significant difference (P = 0.87) in anti-Pv titers between the patients with NHL (mean anti-Pv titer = 1:742) and the controls (mean anti-Pv titers = 1:866) at the end of the observation period.

Adverse reactions to booster immunization. Four patients with NHL and a low anti-Pv titer after primary immunization were revaccinated with 0.5 ml of Pneumovax 23. Two patients developed local erythema at the site of injection, while no adverse reactions were observed in the other two patients.

Antibody response to booster immunization. The mean anti-Pv titer before revaccination (1:159) did not differ significantly from the pretreatment value (1:125). The antibody titers were measured after 1 month (mean anti-Pv titer = 1:496) and 6 months (mean anti-Pv titer = 1:604).
The antibody response to pneumococcal capsular polysaccharide vaccine was evaluated by ELISA with the vaccine as the antigen. Because of the weak interaction of carbohydrates with plastic surfaces, the antigen mixture was derivatized with cyanuric chloride and covalently linked to poly-L-lysine.

Eleven patients with NHL is a low number for a meaningful statistical analysis. Laparotomy, including diagnostic splenectomy, has long been a standard procedure in the staging of NHL. With the availability of high-resolution computer tomography and magnetic resonance imaging on the one hand, and the application of effective cytotoxic drugs on the other, laparotomy is performed only in exceptional cases of NHL nowadays. Even in stage I high-grade NHL, chemotherapy in combination with radiotherapy is advocated, making laparotomy an infrequent procedure with these patients.

Consequently, in patients with NHL, splenectomy is performed only in rare cases: diagnostic splenectomy is performed in patients in whom the spleen is the only area afflicted with lymphoma, and therapeutic splenectomy is performed in patients with symptomatic splenomegaly resistant to chemotherapy or radiation.

In this study, the mean concentration of antibodies against \( S. \) \textit{pneumoniae} prior to immunization was lower in patients with NHL than in patients who had undergone splenectomy for other reasons; however, this difference was not statistically significant. Ballester et al. (5) detected markedly decreased concentrations of antibodies against pneumococcal polysaccharides in unvaccinated patients with NHL but high natural antibody titers in patients with nonneoplastic diseases.

As determined by ELISA with the whole 23-valent vaccine as the antigen, a significant rise in the antibody titer was elicited in 45.4% of the lymphoma patients and in 57.1% of patients with nonneoplastic diseases. The differences in the antibody levels of the two groups were statistically not significant.

It is recommended that a pneumococcal vaccination be delivered 14 days prior to splenectomy. The control patients in this study suffered from idiopathic thrombocytopenic purpura and from hemolytic anemia. Splenectomy in these cases had to be performed due to an emergency (e.g., hemolytic crisis or thrombocytopenic bleeding). Because of these circumstances, the control patients were vaccinated immediately prior to splenectomy. This might explain the lower antibody response to the pneumococcal polysaccharides in our controls than in control patients in other studies. Also, the NHL patients were vaccinated immediately prior to splenectomy. Especially with high-grade NHL, chemotherapy should not be postponed for 2 weeks. Thus, the short period between the vaccination and the splenectomy was common to both the lymphoma patients and the controls in our study.

The antibody responses to individual capsular polysaccharides contained in the vaccine vary widely (8, 15, 24, 29). Since the presence or absence of development of antibodies to a single component of the vaccine is without consequence as far as vaccination is concerned (there is no possibility to reimmunize with single capsular polysaccharides), we decided to evaluate the immunoresponse to Pneumovax 23 by using the derivatized whole-carbohydrate mixture as the antigen in our ELISA.

Patients with a small antibody titer increase after the primary immunization may benefit from revaccination. Borgono et al. (7) observed an increase in antibody levels when booster doses were given to healthy infants who responded insufficiently to the first injection. In the present study, patients who did not respond to primary immunization and patients who were in remission or who had minimal disease after having completed irradiation and chemotherapy were revaccinated.

### TABLE 3. Antibody response to revaccination with \( S. \) \textit{pneumoniae} polysaccharides in splenectomized patients with NHL not responding to primary immunization

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Before booster immunization</th>
<th>5–15 wk after booster immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALT</td>
<td>25</td>
<td>32</td>
</tr>
<tr>
<td>CB-CC</td>
<td>268</td>
<td>231</td>
</tr>
<tr>
<td>Immunocytoma</td>
<td>297</td>
<td>365</td>
</tr>
<tr>
<td>MALT</td>
<td>46</td>
<td>170</td>
</tr>
</tbody>
</table>

\(^a\) Splenectomized patients with NHL not responding to immunization with the pneumococcal vaccine were given a booster dose of the 23-valent pneumococcal polysaccharide vaccine. The patients did not receive multiantigen chemotherapy or irradiation treatment subsequent to revaccination.

\(^b\) MALT, mucosa-associated lymphatic tissue lymphoma; CB-CC, centroblastic-centrocytic lymphoma.

cantly from the mean pneumococcal-antibody titer 5 to 15 weeks after reimmunization (1:199; \( P = 0.7 \); Table 3).

The development of antibodies to cell wall polysaccharide (only measured in the sera of patients with a positive immune response) was found to be weak and was therefore ignored (24).

### DISCUSSION

The increased risk of overwhelming infections with \( S. \) \textit{pneumoniae} in splenectomized patients has led researchers to recommend prophylactic antibiotics for such patients. The demonstration of an adequate antibody response in adults splenectomized because of trauma (1) supports the vaccination of splenectomized patients to protect them against pneumococcal polysaccharides.

Splenectomized patients with Hodgkin’s disease who are injected with pneumococcal polysaccharides after the initiation of cytoreductive therapy show a suboptimal response to the vaccine (25, 34). However, in these patients a significant rise in the level of antibodies was detected when the vaccine was administered prior to irradiation and chemotherapy (2, 13). Splenectomized patients with Hodgkin’s disease are at risk for pneumococcal sepsis even when the pneumococcal vaccines are administered adequately (11, 31).

So far, only limited data on the immunogenic effect of bacterial vaccines in patients with NHL have been published. Ballester et al. (5) found an impaired humoral response in NHL patients vaccinated several months after the administration of cytoreductive therapy. Grimfors et al. (17) vaccinated nine splenectomized NHL patients prior to lymphoma treatment. They report the inadequate formation of antibodies against pneumococcal polysaccharides. In their study a 14-valent vaccine was administered, and the authors evaluated the titer of antibodies against only two capsular antigens of \( S. \) \textit{pneumoniae}, namely, 6A and 19F. In a second study, a group from the same laboratory (18) measured a fairly good antibody response in 25 NHL patients, although some of these patients were being treated with chemotherapy or irradiation at the time of vaccination. In the latter investigation the antibody response was determined 14 days after the immunization.

It was the objective of the present investigation to assess the immunogenicity of a 23-valent pneumococcal vaccine (Pneumovax 23) in splenectomized patients with NHL. Furthermore, patients not responding to primary immunization and who were in remission or who had minimal disease after having completed irradiation and chemotherapy were revaccinated.
ment were revaccinated. These patients proved to be virtually unresponsive to booster doses of polysaccharide vaccines. The finding of disparate antibody responses in patients with NHL following vaccination with pneumococcal polysaccharides has been described previously, and the failure of the four initially unresponsive subjects to respond to revaccination was not unexpected. Many bacterial capsular polysaccharides appear not to be degradable by mammalian enzymes and may remain detectable in lymph nodes with immunofluorescent type-specific antibodies for long periods (22). Responses in healthy subjects, when they do occur, tend to be of low magnitude and short duration.

The results obtained here suggest that vaccination with pneumococcal polysaccharides might help protect against invasive pneumococcal infections in a considerable fraction of splenectomized NHL patients and should therefore be administered. Nevertheless, some subjects with malignant lymphoma do not benefit from immunization. Currently, protein-polysaccharide conjugates of S. pneumoniae that have a higher immunogenicity are being developed (12, 26, 32). However, a study comparing a 23-valent pneumococcal polysaccharide vaccine with a 7-valent pneumococcal-conjugate vaccine with Hodgkin's disease demonstrated a lower response to the 7-valent pneumococcal-conjugate vaccine than to the 23-valent vaccine (27). The difficulties associated with antibody prophylaxis, including the development of bacterial resistance (30) and the short duration of increased levels of antibodies against pneumococci after the infusion of gamma globulins (28), underline the need for improved immunoprophylactic methods.

REFERENCES