Chapter 5

Synergistic immunosuppressive effect of anti-TNF combined with methotrexate on antibody responses to the 23 valent pneumococcal polysaccharide vaccine

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abstract

objective
The efficacy of the immune response upon vaccination in patients treated with anti-tumor necrosis factor-alpha (anti-TNF) with or without methotrexate is subject of debate. We studied the effect of immunosuppressive treatment, including anti-TNF and methotrexate, on the response to pneumococcal polysaccharide (PPS) vaccine.

methods
Fifty two patients treated with immunosuppressives including anti-TNF (anti-TNF group), 41 patients given a similar immunosuppressive regimen without anti-TNF (no anti-TNF group), and 18 healthy controls were vaccinated with a 23 valent PPS vaccine. The percentage of patients treated with methotrexate in the anti-TNF and no anti-TNF group was 65% and 76% respectively. Antibodies against four of the vaccine antigens (PPS 6B, 9V, 19F and 23F) were measured before and four weeks after vaccination. The primary outcome was the response rate, defined as the percentage with a postvaccination titer ≥ 0.35 μg/ml in combination with at least a two-fold increase in antibody titer. The protection rate was defined as a postvaccination titer ≥ 0.35 μg/ml.

results
The use of methotrexate was the strongest predictor of impaired vaccination outcome. Anti-TNF caused an additional immunosuppressive effect in the presence of methotrexate, leading to the lowest response percentages in patients using the combination of these two drugs. The underlying disease, other immunosuppressives such as prednisone or type of anti-TNF agent used did not influence vaccination outcome.

conclusion
Patients who were treated with the combination of methotrexate and anti-TNF demonstrated a significantly impaired immune response following pneumococcal polysaccharide vaccination as compared to patients treated with either methotrexate or anti-TNF only or immunosuppressives excluding these two compounds.
introduction

Anti-tumor necrosis factor-alpha treatment (anti-TNF) is effective in the treatment of autoimmune disorders like rheumatoid arthritis (RA), ankylosing spondylarthropathy, psoriasis and Crohn’s disease(1,2). A growing number of patients are treated with one of three compounds currently registered for clinical usage: infliximab (Remicade™), a chimeric monoclonal antibody against TNF, etanercept (Enbrel™), a soluble TNF receptor, and adalimumab (Humira™), a humanized monoclonal anti-TNF antibody(3-6). Although highly beneficial in the treatment of these autoimmune diseases, blocking the effects of TNF also leads to a specific defect in the cell mediated host immunity, most notably leading to severe infections with intracellular micro-organisms, such as tuberculosis(7-10). The three available compounds differ in the rate at which complications, such as tuberculosis, occur underlining subtle immunological differences in their mode of action(11). Common respiratory tract infections occur 2-4 times more frequent among patients treated with anti-TNF and severe bacterial infections, including invasive pneumococcal disease, have been reported during anti-TNF treatment(12-14). Guidelines indicate that vaccination with the pneumococcal polysaccharide (PPS) vaccine should be considered in rheumatic or inflammatory bowel disease (IBD) patients treated with immunosuppressive medication, including anti-TNF(15-18). The use of immunosuppressives however can reduce the response upon vaccination. We reported earlier that anti-TNF has a modest, but significant, negative impact on the response to the T-cell dependent influenza vaccine(19). Other studies reported no significant negative impact of treatment with anti-TNF on the response upon the pneumococcal polysaccharide vaccine while some have identified methotrexate as an inhibitor of this response(20-25). The aim of the present study is to establish the influence of anti-TNF either alone or in combination with methotrexate on the antibody response upon vaccination with the 23 valent pneumococcal polysaccharide vaccine (PPS23).

methods

subjects

Patients, 18 years of age or older, treated with anti-TNF at the Leiden University Medical Center, The Netherlands, were invited to participate in this open-label study, when visiting either the rheumatology or gastro-enterology outpatient clinic. Pregnancy and an active infectious disease were the only exclusion criteria. From approximately 1000 patients with Crohn’s disease and 2000 patients with rheumatoid arthritis who visit these outpatient clinics a small proportion (<7 %, n = 207) was treated with anti-TNF at the moment when this study was conducted. Thirty three percent (n = 69) of these patients were eligible and willing to participate and were enrolled; fifty two patients treated with anti-TNF completed both study visits (anti-TNF group). Patients not treated with anti-TNF were selected from the same outpatient clinics to match those treated with anti-TNF for sex, age and immunosuppressive regimen, 54 patients were enrolled, of whom 41 completed both study visits (no anti-TNF group). Eighteen healthy controls, recruited through advertisements, matched for sex and age, completed two study visits.
The main reason for premature drop out from this study was the inability to make extra visits because of the distance to the clinic or because of lack of time. None of the subjects received prior pneumococcal vaccination. The protocol was designed according to the good clinical research guidelines and approved by the medical ethical committee (MEC) of the Leiden University Medical Center (local MEC number P03.144) and a written informed consent was provided by all subjects.

**vaccine**
The 23 valent pneumococcal polysaccharide (PPS23) vaccine used (0.5 ml Pneumo-23®, Merck Sharp and Dohme, Haarlem, The Netherlands) which contained 25 μg of each of the following capsular polysaccharides: type 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F (Danish nomenclature). Vaccines were stored at 6 °C and administered at room temperature. Vaccinations were given according to the package insert by intra-muscular injection in the right deltoid muscle, four weeks after an influenza vaccination. Patients registered side effects in a study log.

**antibody assays**
Serum samples for determination of pneumococcal antibodies were obtained at the study visits at week 0 (before the vaccination) and 4 weeks thereafter and were stored at – 80 °C until analysis.

Post vaccination IgG antibody levels to four pneumococcal serotypes 6B, 9V, 19F and 23F were measured by ELISA as described previously. (26) All sera were pre-incubated overnight at 4 °C with pneumococcal cell wall polysaccharide (CPS) in diluting buffer for blocking of non-specific anti-CPS antibodies (50 μg/ml; Statens Serum Institute, Copenhagen, Denmark). The pneumococcal antibody reference serum (lot 89-SF) was used for assay standardisation.

**primary outcome and statistics**
The response rate was defined, in line with World Health Organisation recommendations, as the percentage with a postvaccination antibody titer ≥ 0.35 μg/ml in combination with at least a two-fold increase in antibody titer. (27) The protection rate was defined as the percentage with a postvaccination antibody titer ≥0.35 μg/ml, irrespective of the antigen used, even though some polysaccharides are known to illicit better responses than others. Geometric mean titers (GMTs) are reported to provide more insight into these differences.

The results were analyzed by one-way ANOVA for GMTs. Response rates were compared using Mantel-Haenszel common odds ratio’s and a two-sided χ²-test. A p-value <0.05 was considered to indicate a statistical significant difference between groups. A backward stepwise elimination using a logistic regression model incorporating the variables anti-TNF, methotrexate, underlying disease, age and sex was used for analysis. Variables that showed no significant impact on vaccination outcomes (the use of prednisone or azathioprine, time on anti-TNF, time on immunosuppressive therapy and duration of disease) were not further evaluated. Calculations were performed using SPSS for Windows, version 14.0.
Results

Baseline Characteristics

Ninety-three patients (70% female, mean age 49 years, range 18 - 83) and 18 healthy controls (78% female, mean age 47 years, range 21 - 75) were evaluated (table 1). Of these 93 patients 80% had a rheumatologic disease (mostly chronic RA) and 20% had inflammatory bowel disease (mostly chronic Crohn’s disease) as underlying disease. All patients were treated with immunosuppressive drugs such as methotrexate, prednisone or azathioprine; 52 of them (56%) were currently treated with anti-TNF, or had been so in the 2 months prior to study entry (anti-TNF group). On average patients had been treated with anti-TNF for 23 months (range 1.5 - 79 months). The remaining 41 patients received similar immunosuppressive therapy but no anti-TNF (no anti-TNF group) (table 1). The patient groups did not significantly differ in age, sex, time on treatment or the use of immunosuppressive drugs (other than anti-TNF). The percentage of patients with rheumatic diseases was significantly higher in the anti-TNF group compared to the no anti-TNF group. Within the anti-TNF group all three currently available compounds were used, infliximab by 26 patients (50%), etanercept by 10 (19%) and adalimumab by 16 (31%).

Table 1. Baseline characteristics of study subjects.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Anti-TNF Group (n = 52)</th>
<th>No Anti-TNF Group (n = 41)</th>
<th>Healthy Controls (n = 18)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women (%)</td>
<td>71</td>
<td>68</td>
<td>78</td>
<td>ns *</td>
</tr>
<tr>
<td>Age, mean (range), years</td>
<td>50 (23 – 73)</td>
<td>47 (18 – 83)</td>
<td>47 (21 – 75)</td>
<td>ns †</td>
</tr>
<tr>
<td>Rheumatic disease (%)</td>
<td>88</td>
<td>71</td>
<td>-</td>
<td>0.038 ‡</td>
</tr>
<tr>
<td>Duration of disease activity, mean (months)</td>
<td>149</td>
<td>94</td>
<td>-</td>
<td>0.018 §</td>
</tr>
<tr>
<td>Prednisone, % (mean dose, mg)</td>
<td>21 (6)</td>
<td>22 (9)</td>
<td>-</td>
<td>ns ‡</td>
</tr>
<tr>
<td>Methotrexate, % (mean dose, mg)</td>
<td>65 (17)</td>
<td>76 (16)</td>
<td>-</td>
<td>ns §</td>
</tr>
<tr>
<td>Azathioprine, % (mean dose, mg)</td>
<td>10 (115)</td>
<td>15 (108)</td>
<td>-</td>
<td>ns §</td>
</tr>
</tbody>
</table>

* X²-square test (2-sided) for anti-TNF versus no anti-TNF versus healthy controls
† One-way ANOVA for anti-TNF versus no anti-TNF versus healthy controls
‡ X²-square test (2-sided) for anti-TNF versus no anti-TNF
§ Wilcoxon rank sum test (2-sided) for anti-TNF versus no anti-TNF

TNF: tumor necrosis factor alpha; GE: gastro-enterologic; NSAIDs: non-steroidal anti-inflammatory drugs

Chapter 5
geometric mean titers
Vaccination resulted in a significant rise of GMTs for all four polysaccharides in all study groups. Postvaccination geometric mean titers against the four pneumococcal polysaccharides did not significantly differ between the three study groups (figure 1). There was a trend for higher prevaccination titers in the anti-TNF group as compared to both the no anti-TNF group and healthy controls.

figure 1. Geometric mean titer (GMT) pre- and postvaccination (error bars indicate 95% confidence interval).

response rates
A backward stepwise elimination using a logistic regression model analysis incorporating the variables anti-TNF and methotrexate, the interaction of these two drugs, type of anti-TNF, sex, age and the type of underlying disease (gastro-enterologic vs.
rheumatologic) was used to control for confounding. The covariates for sex, age, type of anti-TNF and type of underlying disease were insignificant and hence removed from the final model since only the variables methotrexate, anti-TNF and the interaction of anti-TNF and methotrexate were predictors of outcome. Therefore results are presented in a two-by-two format based on the use of these two drugs. Adjusted odds ratio’s for the use of anti-TNF (vs. no anti-TNF) and methotrexate (vs. no methotrexate) for PPS 6B, 9V and 23F are reported in figure 2. For PPS 19F there was a significant interaction between anti-TNF and methotrexate, thus separate odds ratio’s within subgroups are reported: the immune response in patients using both drugs was significantly inhibited as compared to the use of either drug alone (figure 2).

figure 2. Response rates, defined as the percentage with a postvaccination titer ≥ 0.35 μg/ml in combination with at least a two-fold increase in antibody titer to PPS 6B, 9V, 19F and 23F by use of anti-TNF (TNF) and methotrexate (MTX): + denotes use, - denotes no use.

Reported are statistically significant (and borderline significant) Mantel-Haenszel common odds ratio’s (95% confidence interval) and p-value for anti-TNF vs. no anti-TNF and methotrexate vs. no methotrexate (PPS 6B, 9V and 23F).

Because of a statistical significant interaction of methotrexate and anti-TNF the analysis of the effects of these drugs on the response upon PPS 19F was analysed in the separate subgroups (no anti-TNF or methotrexate, n=18; anti-TNF only, n=10; methotrexate only, n=34; both anti-TNF and methotrexate, n=31). The odds ratio’s that were statistically significant are given: * only methotrexate and ** only anti-TNF vs. both anti-TNF and methotrexate.
From all immunosuppressives evaluated within this study, the use of methotrexate was the strongest predictor of a poor vaccination outcome, reducing the percentage of responders upon pneumococcal polysaccharide vaccination by four- to eleven-fold, depending on the PPS type. A significant inhibiting effect of anti-TNF could only be established in patients using methotrexate (PPS 19F). For PPS 9V and 23F the use of anti-TNF reduced the number of responders by half, an effect that was not statistically significant.

Although subjects on average did respond to PPS 6B (as measured by increase of GMT or the percentage with at least a two-fold titer increase), postvaccination titers were low for this polysaccharide. This resulted in response rates ≤11%, which was too low to detect any significant inhibiting effect of immunosuppressive medication. Healthy controls yielded response rates almost equal to patients not using anti-TNF; patients treated with anti-TNF showed a trend towards lower response rates compared to patients not treated with anti-TNF (table 2).

Table 2. The response rate (RR), defined as the percentage with a postvaccination titer ≥ 0.35 μg/ml in combination with at least a two-fold increase in antibody titer and the protection rate (PR), defined as the percentage with a postvaccination titer ≥ 0.35 μg/ml, per group.

<table>
<thead>
<tr>
<th>PPS</th>
<th>anti-TNF group (n = 52)</th>
<th>no anti-TNF group (n = 41)</th>
<th>healthy controls (n = 18)</th>
<th>P-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR 6B</td>
<td>6</td>
<td>10</td>
<td>6</td>
<td>ns</td>
</tr>
<tr>
<td>PR 6B</td>
<td>15</td>
<td>24</td>
<td>17</td>
<td>ns</td>
</tr>
<tr>
<td>RR 9V</td>
<td>44</td>
<td>99</td>
<td>67</td>
<td>ns</td>
</tr>
<tr>
<td>PR 9V</td>
<td>100</td>
<td>100</td>
<td>94</td>
<td>ns</td>
</tr>
<tr>
<td>RR 19F</td>
<td>14</td>
<td>34</td>
<td>44</td>
<td>p=0.012</td>
</tr>
<tr>
<td>PR 19F</td>
<td>94</td>
<td>93</td>
<td>83</td>
<td>ns</td>
</tr>
<tr>
<td>RR 23F</td>
<td>35</td>
<td>44</td>
<td>39</td>
<td>ns</td>
</tr>
<tr>
<td>PR 23F</td>
<td>83</td>
<td>83</td>
<td>83</td>
<td>ns</td>
</tr>
</tbody>
</table>

* PPS: pneumococcal polysaccharide; TNF: tumor necrosis factor; ns: not significant

$X^2$ test (2-sided) for anti-TNF versus no anti-TNF versus healthy controls

**Protection rates**

Although individual cut off values for the separate polysaccharides would probably give a more accurate representation, no such values have been defined and tested in clinical practice. The general cut off titer of 0.35 μg/ml was easily met by almost all subjects for 3 out of 4 antigens, even in patients treated with both anti-TNF and methotrexate (100, 94 and 79% for PPS 9V, 19F and 23F respectively). Although these percentages were considerably lower for PPS 6B (15% for patients treated with both anti-TNF and methotrexate), this percentage was comparable to the other patient groups and healthy controls for PPS 6B (table 2).
adverse events
Mild reactions, such as pain, were frequently reported after vaccination with no differences between groups. One 41 year old female RA patient, treated with infliximab combined with prednisone, presented herself 48 hours after vaccination with a swelling of the right upper arm, fever and chills, most likely a hyper immune reaction, which was qualified as a severe adverse event, however there were no sequellae. She had a remarkable twenty fivefold increase of antibodies against PPS 9V. No other severe adverse events were reported.

discussion
The main finding of the present study is the synergistic immunosuppressive effect of combined methotrexate and anti-TNF use on the antibody response upon pneumococcal polysaccharide vaccination. In patients receiving both drugs response rates were low (<30% for PPS 9V and 23F), and even almost absent (3%) for both PPS 6B and 19F. This effect was similar with all three currently available anti-TNF agents. Response rates in patients not using the combination of anti-TNF and methotrexate (either drug alone or other immunosuppressive medication) were comparable to the response rates of healthy controls.

Our study consisted of a heterogenic patient group, which mirrors daily practice, however all clinical variables other than the aforementioned did not significantly influence vaccination outcomes. Our findings were consistent for all four polysaccharides tested, even though the mean pre- and postvaccination titers differed substantially between these four polysaccharides. We believe that individual cut-off levels for the different polysaccharides could be more relevant than a universal cut-off of 0.35 μg/ml as suggested by WHO.

We previously reported a significant inhibiting effect of anti-TNF on the antibody response upon influenza vaccination, a T-cell dependent vaccine(19). Combined with the type of opportunistic diseases seen during treatment with anti-TNF, this led to the hypothesis that anti-TNF predominantly influences T-cell dependent immune responses. Vaccination with a polysaccharide vaccine is classically considered to be a T-cell independent process, however, our group and others have shown that some of the capsular types (e.g. PPS 18C, 19F, 23F) clearly illicit an immune response dependent on the presence and functioning of CD4-positive T-lymphocytes (the so called partially T-cell independent, type 2 (TI-2) response), this in contrast with other capsular types (e.g. PPS 1, 4, 6B, 9V, 14) that act as true T-cell independent antigens, eliciting a TI-1 response(28-29). In the present study no clear differences between (expected) TI-1 and TI-2 responses could be identified, although the only response that was significantly inhibited by anti-TNF (upon PPS19F) is partially T-cell dependent. Another study, which included 7 serotypes in the analysis but not PPS 19F found a statistically significant negative impact of anti-TNF on the response to the partially T-cell dependent antigen PPS 23F(20).

The complexity of the immune system is underscored by the fact that we did not find the synergistic effect of methotrexate and anti-TNF when analyzing the immune res-
ponse upon influenza vaccination, within the same cohort(19). This could also mean that the immune suppressive effect of the combination of methotrexate and anti-TNF, also acts on processes unique for the T-cell independent polysaccharides. Some of these B-cell specific (T-cell independent) processes (binding, signaling) have been shown to be TNF dependent and might be inhibited by anti-TNF(30). Combined with the suppression of B-cell function and the subsequent inhibition of antibody production by methotrexate, this might create a specific B-cell defect, beneficial for the treatment of RA but simultaneously leading to impaired immune responses upon polysaccharide exposure(31,32). The synergistic action of anti-TNF and methotrexate has clearly been established in clinical trials assessing different treatment strategies(33). Further evidence that specific B-cell inhibition is relevant in the treatment of RA comes from trials assessing anti-B-cell therapies (e.g. rituximab, belimumab as well as sulfasalazine and its metabolites)(34-38).

Several studies, most of which found no immunosuppressive effect of anti-TNF, have been conducted in patients with rheumatic diseases. In some of these studies patients were vaccinated directly after initiation of anti-TNF treatment, when the immunosuppressive effect might not have been fully established(22,23). Whether or not patients with early RA demonstrate the same level of immunosuppression as chronically ill patients is not known(25) The patients in the present study had longstanding disease and were treated with anti-TNF on average for almost two years. The trend for higher prevaccination titers found in the present study, in patients chronically treated with anti-TNF, might represent a higher pneumococcal infection or colonization rate. The percentage of patients who were treated for less than three months with anti-TNF in the present study was too small to calculate the effect of a shorter exposure to these drugs. Higher prevaccination titers did not inhibit the antibody response upon vaccination in the present study, they were actually correlated with higher postvaccination titers in all study groups.

A study by Kapetanovic et al., performed in a study population resembling the population of the present study, also identified methotrexate as the major inhibitor of the immune response upon pneumococcal polysaccharide vaccination(21) This effect paradoxically seemed to be reversed by the use of anti-TNF in their study. Because of differences in study design, patient populations and outcome measurements the comparability of the present study to other vaccination studies conducted in patients treated with anti-TNF is limited. The differences found, stress the need for large, well designed vaccination trials in immunocompromised patients.

The true clinical efficacy of pneumococcal vaccination (a reduction of invasive disease and mortality attributable to pneumococcal infections) can only be established in trials including thousands of participants. Since this is not feasible for patients treated with anti-TNF, surrogate markers, such as the response rate, are the best estimate of clinical efficacy. The fact that we found blunted response rates in patients treated with the combination of methotrexate and anti-TNF, should not be used to exclude these patients from initiatives that promote vaccine uptake in immunocompromised patients(39-41). Even in these ‘worst responders’ we did find antibody titer increases upon vaccination and high postvaccination titers, which are also correlated with protection from invasive pneumococcal disease(27) This study was not designed to
evaluate the value of routinely measuring postvaccination titers in these patients. We conclude that the response upon the pneumococcal polysaccharide vaccine is significantly impaired in patients treated with methotrexate, especially when methotrexate is combined with anti-TNF. However, there are no arguments to withhold pneumococcal vaccination from this subgroup of patients with combined methotrexate and anti-TNF therapy. Ideally, patients should receive pneumococcal vaccination before immunosuppressive therapy with methotrexate and/or anti-TNF is initiated or intensified.
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