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Immunizations in immunocompromised hosts : effects of immune modulating drugs and HIV on the humoral immune response

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Immunizations in immunocompromised hosts:

**effects of immune modulating drugs and HIV on the
humoral immune response**

Luc Ben Stefan Gelinck

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26__ during the conduction of the RICH2 trial (neon adaptation of photograph). Photo &
27__ design: Luc Gelinck.

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29__

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3__ **Immunizations in immunocompromised hosts:**
4__ effects of immune modulating drugs and HIV on the
5__ humoral immune response
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13__ **Proefschrift**
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19__

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22__

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24__

25__ **Luc Ben Stefan Gelinck**
26__ geboren te Nijmegen in 1970
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1___ *Quid nobis certius ipsis sensibus esse potest, qui vera ac falsa notemus?*

2___ Lucretius (99-55 v. Chr.)

3___ De Rerum Natura (liber primus)

4___ 699-700

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32___ What better than the senses can enable us to distinguish the false from the true?

33___ Wat geeft meer zekerheid dan de zintuigen, om onderscheid te maken tussen

34___ waar en onwaar?

35___ *Titus Lucretius Carus, 'over de natuur der dingen', eerste boek*

36___

37___ Deze zinsnede van Lucretius werd ook door Edward Jenner in 1798 als opdracht opgenomen in zijn werk over

38___ variolisatie, de eerste wetenschappelijke publicatie over vaccinatie (referentie 2 van de 'General introduction' van

39___ dit proefschrift).

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Introduction

General introduction

Thesis

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*Dept. of Infectious Diseases, Leiden University Medical Center (LUMC), Leiden and
Dept. of MMI, Erasmus Medical Center, Rotterdam, The Netherlands*

1 GENERAL INTRODUCTION

2

3 History of vaccination

4 In the course of the past centuries vaccination has been proven to be one of the most
5 beneficial interventions made in medicine. Vaccination and improved sanitation ended
6 the status of infectious diseases as a leading cause of death in many parts of the world,
7 thereby saving more lives than any other medical innovation. [1]

8 Inoculation and variolation were common practice in large parts of the world already
9 for centuries, in an attempt to protect people from smallpox. Edward Jenner's 1798
10 publication on variolation however is the first scientific report on vaccination. [2] Some
11 80 years later Pasteur published on live attenuated micro-organisms which provided im-
12 munity against a challenge with virulent micro-organisms, which led to his statement that
13 vaccination was now ready for human use. Many disagreed with him on this point, even
14 when he successfully vaccinated Joseph Meister, suffering from rabies, with a chemically
15 attenuated rabies strain in 1885. The development of safer, killed, vaccines followed shortly
16 after these experiments and led to vaccines against Typhoid, Cholera and Plague before
17 the beginning of the 20th century. Mass vaccination campaigns held in the British army
18 were frustrated by suspicion and emotional opposition, which led to an excess mortality
19 of 9000 (unvaccinated) British soldiers due to typhoid fever before the first World War.

20 Vaccine research flourished in the 20th century: vaccines were developed for more than
21 20 pathogens, with a clear decreased circulation of many of those pathogens in the general
22 population. Complete eradication of infectious diseases by vaccination has been shown an
23 almost impossible quest. The natural circulation of variola, the virus that causes smallpox,
24 was interrupted by intensive vaccination campaigns held by the World Health Organisa-
25 tion (WHO) in 1974. Although efforts to try and eradicate polio are not without success,
26 the WHO has had to move the date of expected eradication of this viral disease forward,
27 more than once. The fact that the history of vaccination does not only consist of success
28 stories is underlined by the difficulties to produce an effective vaccine against the human
29 immunodeficiency virus (HIV).

30 Despite the undisputed merits of vaccination, the negative perception of the general
31 public has never completely subsided since the first experiments of Jenner and Pasteur.
32 Although there is growing, solid evidence that for instance autism is not caused by the
33 vaccine adjuvant thiomersal, some people still refrain their children from vaccination
34 because of this fear. [3]

35

36 Immunology of vaccination

37 Although the complexity of the humane immune system was almost completely unre-
38 vealed in his time, Jenner realized as early as in 1810 that immunity against smallpox was
39 not lifelong and that revaccination might be necessary.

Even now we do not understand all immunological processes involved in the immune response upon vaccination in detail. [4] Molecular techniques such as crystallography, genome wide association scans and cell marker studies did advance our understanding of vaccine immunology today. However, in clinical registration trials, it is still the clinical endpoint (preventing severe disease or death due to a certain pathogen) that has to prove the efficacy of a vaccine.

The immune system is a complex interplay of cellular and soluble factors with the combined 'goal' of protecting the host against potentially dangerous factors (such as toxins or micro-organisms). Both micro-organisms and the immune system have become extremely sophisticated over ages of co-evolution.

Figure 1. shows a schematic, over-simplified, representation of human immunology. Mechanic barriers, and processes like mucus, teardrops and urine production, help to protect humans from exogenous micro-organisms. Once past these barriers, pathogens will be recognized immediately by the 'innate' immune system, which is able to respond on numerous pathogens. Granulocytic cells and inflammatory molecules act synergistically, stimulating each other in order to respond to a 'threat'. This response is rather aspecific and can be both local or systemic. Tumor necrosis factor alpha is an important intermediary molecule which orchestrates this immune response. There is no immunological memory formed upon an innate immune response. This is in contrast with a more specific immune response, the adaptive immune response, which is able to form a memory for the antigens it encountered, making the response upon a second confrontation with the same antigen (booster response) faster, stronger and more specific. The processes involved in forming such a booster response are typically T-cell-dependent and include a class switch from IgM to IgG antibodies, the forming of a booster reaction (a logarithmic titer increase within

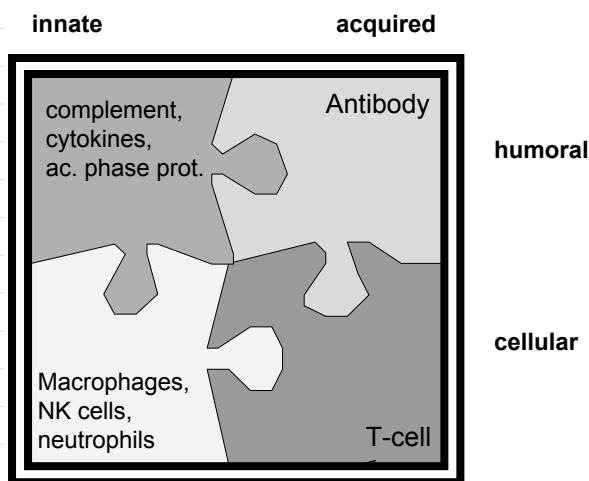


Figure 1. Schematic representation of human immunology.

1 days after re-exposure to the antigen) and avidity maturation (clonal selection of the most
 2 specific antibodies). An adaptive immune response is initiated only after an antigen is
 3 processed and presented to T-lymphocytes by antigen-presenting cells. Stimulation of
 4 the effector cells takes place after specific receptor and co-receptor binding resulting in a
 5 response for each unique antigen.

6 The goal of vaccination is to 'prime' the immune system for a certain, specific, antigen. The
 7 immune response upon vaccination typically includes antibody production; most vaccines
 8 will also induce a T-cell response, leading to a long lasting memory against the pathogen.
 9 Both antibodies and specific antibody producing B-cell might persist for many decades. [5]

10 In some cases (e.g. rabies, hepatitis A and B vaccines) immunization will lead to protec-
 11 tive immunity. In other cases (e.g. pneumococcal and influenza vaccines) there will not be
 12 protective immunity in all who are vaccinated, still the immunity elicited by vaccination
 13 will most likely mitigate the course of a disease.

14 Figure 2 depicts the two main routes of antibody production upon vaccination. T-
 15 cell-independent (vaccine) antigens (typically polysaccharides) bind and cross-link B-cell
 16 antigen receptors, stimulating the B-cell to produce antibodies against this antigen,
 17 without the formation of memory. T-cell-dependent antigens are processed and presented
 18 by antigen producing cells, which initiate the immune response. In this thesis we used
 19 both a T-cell-independent vaccine (polyvalent pneumococcal polysaccharide vaccine) and
 20 T-cell-dependent vaccines (influenza, rabies) to investigate both immunological pathways.

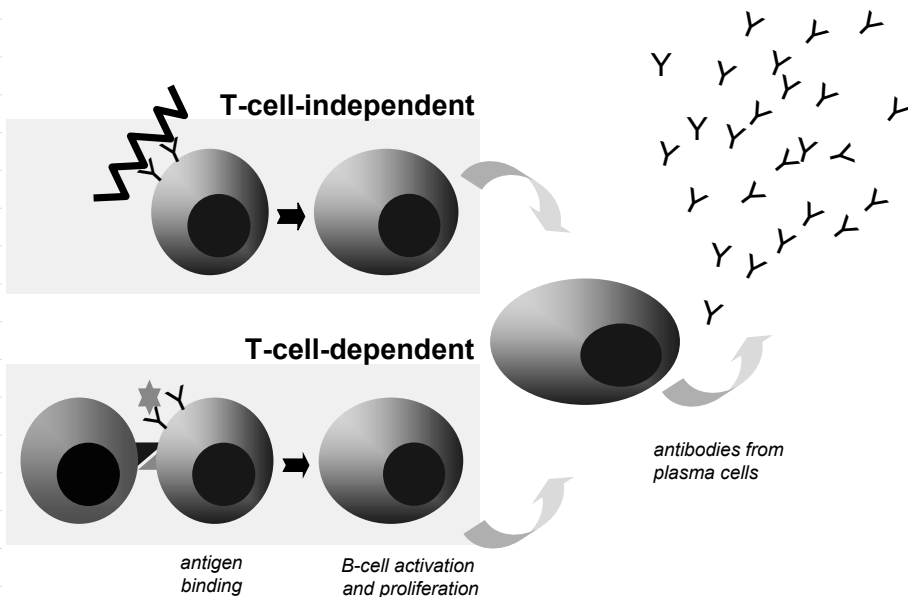


Figure 2. The two main routes of antibody production upon vaccination: T-cell-independent and T-cell-dependent immune responses.

1__ Current vaccination practices

2__ The Dutch National Vaccine Program is well embedded in the national healthcare system.
 3__ More than 90 percent of all children are immunized according to a national schedule,
 4__ which is updated regularly. [6] Only a small percentage of children is willingly not vacci-
 5__ nated, mostly on religious grounds. Besides vaccines against diphtheria, tetanus, pertusis,
 6__ polio, mumps, measles and rubella the national vaccine program now also includes vac-
 7__ cines against *Haemophilus influenzae* type B, *Neisseria meningitidis* group C, pneumocci
 8__ and human papilloma virus.

9__ Some vaccines, such as hepatitis B, are indicated in selected risk groups only. Influenza
 10__ vaccination is indicated for everybody with an age of 60 years or older; diabetics and
 11__ cardio-pulmonary compromised patients. For patients with an impaired immunity the
 12__ Dutch guidelines state that influenza and pneumococcal vaccination should be consid-
 13__ ered. [7,8]

15__ Immunocompromised patients

16__ A growing number of patients lives with an immunodeficiency acquired by disease or
 17__ medical intervention. Several groups of patients, with different mechanisms of the im-
 18__ paired immunity, can be identified. Three groups of immunocompromised patients are
 19__ represented in this thesis.

- 20__ 1. Patients treated with immune modulating medication in order to suppress the activity
21__ of auto-immune disorders.
- 22__ 2. Patients infected with HIV.
- 23__ 3. Patients who underwent a hematological stem cell transplantation, mostly because of
24__ a hematological malignancy.

25__ These three patient groups represent a wide variety of different mechanisms of immune
 26__ impairment. For some diseases or immune modulating drugs the effect on the immunity
 27__ and more specific the immune response upon vaccination is well characterized. Untreated
 28__ HIV for instance ultimately causes a T-cell deficiency, which can be reversed by adequately
 29__ suppressing HIV replication. The immune deficiency caused by chronic HIV infection
 30__ extends beyond the T-cells and in part also affects humoral immunity.

31__ For new immune modulating drugs, the impact on immunity is often not well charac-
 32__ terized. Vaccination studies may help to characterize the immunological consequences of
 33__ drugs like anti-TNF. Comparing the outcomes in the different groups of patients helps to
 34__ weigh the severity of the immunodeficiency.

35__ In this thesis we mainly report antibody responses upon vaccination. By combining the
 36__ use of T-cell-dependent and T-cell-independent vaccines, conclusions can be drawn about
 37__ the immunological processes that result in antibody formation.

38__
 39__

1__ Vaccination of immunocompromised patients

2__ Vaccination of immunocompromised patients is more complex than vaccination of
3__ healthy subjects for several reasons. The immune deficiency places patients more at risk for
4__ infectious diseases, raising the importance of adequate protection. The goal of protection
5__ is more difficult to achieve since the immune impairment is likely to interfere with the
6__ immune response. One of the goals of the studies described in this thesis was to explore
7__ a strategy (intradermal vaccination) that might increase the response upon vaccination
8__ in these patients. If patients who do not properly respond upon vaccination are timely
9__ recognized, alternative measures can be taken for protection.

10__
11__ Studies as described in this thesis increase our understanding of the immunology and
12__ protection against potentially fatal diseases in those who are the most at risk.

13__

14__

15__ **OUTLINE OF THE THESIS**

16__

17__ The focus of this thesis is the immune response upon vaccination in immunocompromised
18__ patients. Three subjects form the core of this thesis.

19__

20__ *1. The effect of new immune modulating drugs on the antibody response upon vaccination*

21__ The studies described in the first two chapters of this thesis originated from a simple
22__ question from a clinician who wondered about the immune response upon vaccination in
23__ a patient treated with anti-TNF. At that time, the spring of 2003, that question proved
24__ impossible to answer, since no data were published on that subject. To be able to answer
25__ this question, a clinical trial, with the acronym RICH1 for the immune Response in Im-
26__ munoCompromised Hosts (1), incorporating a T-cell-independent and a T-cell-dependent
27__ vaccine, was designed. In the study described in **chapter 1** the effect of anti-TNF (with
28__ or without methotrexate) on the response to pneumococcal vaccination is discussed and
29__ in **chapter 2** the effect on influenza vaccination. The **appendix to chapter 1 and 2** shows
30__ data from the RICH1 study that were not published before. **Chapter 3** describes the
31__ effects of another immune modulating agent which is increasingly being used in patients
32__ failing anti-TNF: rituximab, a B-lymphocyte antagonist.

33__

34__ *2. Intradermal vaccination in immunocompromised patients*

35__ The chapters 3, 4 and 5 stem from the RICH2 study, which was conducted 2 years after
36__ the RICH1 study. In the study described in **chapter 4** we explored intradermal influenza
37__ vaccination, to try and improve vaccination outcomes in immunocompromised patients.
38__ Here too, unpublished additional data, including long-term follow-up data, are included
39__ in the **appendix to chapter 3 and 4**.

3. *Special aspects of the immune response upon vaccination in HIV-infected adults*

The study described in **chapter 5** explores the ability of immunocompromised patients to form broad reacting anti-bodies upon influenza vaccination.

In the study described in **chapter 6** a more in depth analysis of several immunological aspects of the immune response upon rabies vaccination in HIV-infected patients is described. By administering a T-cell-dependent neo antigen in a group of HIV-infected individuals with low CD4 nadir counts before the initiation of antiretroviral treatment, conclusions can be drawn about the functioning the T-cells that are newly formed in the process of immune reconstitution that follows the treatment of HIV. The study cohort described in this chapter is unique in the fact that most patients were followed up from before the antiretroviral treatment era (allowing for the low CD4 nadir counts) and all subjects could be evaluated 5 years after vaccination.

The current vaccination practice in HIV-infected adults is reviewed in **chapter 7**.

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Chapter | 1

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

Vaccine 2008;26:3528-33.

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1 ABSTRACT

2
3 **Introduction.** The efficacy of the immune response upon vaccination in patients treated
4 with anti-tumor necrosis factor-alpha (anti-TNF) with or without methotrexate is subject
5 of debate. We studied the effect of immunosuppressive treatment, including anti-TNF
6 and methotrexate, on the response to pneumococcal polysaccharide (PPS) vaccine.

7 **Methods.** Fifty two patients treated with immunosuppressives including anti-TNF (anti-
8 TNF group), 41 patients given a similar immunosuppressive regimen without anti-TNF
9 (no anti-TNF group), and 18 healthy controls were vaccinated with a 23 valent PPS
10 vaccine. The percentage of patients treated with methotrexate in the anti-TNF and no
11 anti-TNF group was 65% and 76% respectively. Antibodies against four of the vaccine
12 antigens (PPS 6B, 9V, 19F and 23F) were measured before and four weeks after vac-
13 cination. The primary outcome was the response rate, defined as the percentage with a
14 postvaccination titer ≥ 0.35 $\mu\text{g/ml}$ in combination with at least a two-fold increase in
15 antibody titer. The protection rate was defined as a postvaccination titer ≥ 0.35 $\mu\text{g/ml}$.

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

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

1 INTRODUCTION

2
3 Anti-tumor necrosis factor-alpha treatment (anti-TNF) is effective in the treatment of
4 autoimmune disorders like rheumatoid arthritis (RA), ankylosing spondylarthropathy, psor-
5 iasis and Crohn's disease. [1,2] A growing number of patients are treated with one of three
6 compounds currently registered for clinical usage: infliximab (Remicade™), a chimeric
7 monoclonal antibody against TNF, etanercept (Enbrel™), a soluble TNF receptor, and
8 adalimumab (Humira™), a humanized monoclonal anti-TNF antibody. [3-6] Although
9 highly beneficial in the treatment of these autoimmune diseases, blocking the effects of
10 TNF also leads to a specific defect in the cell mediated host immunity, most notably
11 leading to severe infections with intracellular micro-organisms, such as tuberculosis. [7-
12 10] The three available compounds differ in the rate at which complications, such as
13 tuberculosis, occur underlining subtle immunological differences in their mode of action.
14 [11] Common respiratory tract infections occur 2-4 times more frequent among patients
15 treated with anti-TNF and severe bacterial infections, including invasive pneumococcal
16 disease, have been reported during anti-TNF treatment. [12-14] Guidelines indicate that
17 vaccination with the pneumococcal polysaccharide (PPS) vaccine should be considered in
18 rheumatic or inflammatory bowel disease (IBD) patients treated with immunosuppressive
19 medication, including anti-TNF. [15-18] The use of immunosuppressives however can
20 reduce the response upon vaccination. We reported earlier that anti-TNF has a modest,
21 but significant, negative impact on the response to the T-cell-dependent influenza vaccine.
22 [19] Other studies reported no significant negative impact of treatment with anti-TNF on
23 the response upon the pneumococcal polysaccharide vaccine while some have identified
24 methotrexate as an inhibitor of this response. [20-25]

25 The aim of the present study is to establish the influence of anti-TNF either alone or in
26 combination with methotrexate on the antibody response upon vaccination with the 23
27 valent pneumococcal polysaccharide vaccine (PPS23).

30 METHODS

32 Subjects

33 Patients, 18 years of age or older, treated with anti-TNF at the Leiden University Medi-
34 cal Center, The Netherlands, were invited to participate in this open-label study, when
35 visiting either the rheumatology or gastro-enterology outpatient clinic. Pregnancy and
36 an active infectious disease were the only exclusion criteria. From approximately 1000
37 patients with Crohn's disease and 2000 patients with rheumatoid arthritis who visit these
38 outpatient clinics a small proportion (<7%, n = 207) was treated with anti-TNF at the
39 moment when this study was conducted. Thirty three percent (n = 69) of these patients

1__ were eligible and willing to participate and were enrolled; fifty two patients treated with
 2__ anti-TNF completed both study visits (anti-TNF group). Patients not treated with anti-
 3__ TNF were selected from the same outpatient clinics to match those treated with anti-TNF
 4__ for sex, age and immunosuppressive regimen, 54 patients were enrolled, of whom 41
 5__ completed both study visits (no anti-TNF group). Eighteen healthy controls, recruited
 6__ through advertisements, matched for sex and age, completed two study visits.

7__ The main reason for premature drop out from this study was the inability to make extra
 8__ visits because of the distance to the clinic or because of lack of time. None of the subjects
 9__ received prior pneumococcal vaccination.

10__ The protocol was designed according to the good clinical research guidelines and ap-
 11__ proved by the medical ethical committee (MEC) of the Leiden University Medical Center
 12__ (local MEC number P03.144) and a written informed consent was provided by all subjects.

13__

14__ Vaccine

15__ The 23 valent pneumococcal polysaccharide (PPS23) vaccine used (0.5 ml Pneumo-23®,
 16__ Merck Sharp and Dohme, Haarlem, The Netherlands) which contained 25 µg of each of
 17__ the following capsular polysaccharides: type 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A,
 18__ 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F (Danish nomenclature). Vac-
 19__ cines were stored at 6°C and administered at room temperature. Vaccinations were given
 20__ according to the package insert by intra-muscular injection in the right deltoid muscle,
 21__ four weeks after an influenza vaccination. Patients registered side effects in a study log.

22__

23__ Antibody assays

24__ Serum samples for determination of pneumococcal antibodies were obtained at the study
 25__ visits at week 0 (before the vaccination) and 4 weeks thereafter and were stored at – 80°C
 26__ until analysis.

27__ Post vaccination IgG antibody levels to four pneumococcal serotypes 6B, 9V, 19F and
 28__ 23F were measured by ELISA as described previously. [26] All sera were pre-incubated
 29__ overnight at 4°C with pneumococcal cell wall polysaccharide (CPS) in diluting buffer
 30__ for blocking of non-specific anti-CPS antibodies (50 µg/ml; Statens Serum Institute,
 31__ Copenhagen, Denmark). The pneumococcal antibody reference serum (lot 89-SF) was
 32__ used for assay standardisation.

33__

34__ Primary outcome and statistics

35__ The response rate was defined, in line with World Health Organisation recommendations,
 36__ as the percentage with a postvaccination antibody titer ≥ 0.35 µg/ml in combination with
 37__ at least a two-fold increase in antibody titer. [27] The protection rate was defined as the
 38__ percentage with a postvaccination antibody titer ≥ 0.35 µg/ml, irrespective of the antigen

39__

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 ratios and a two-sided χ^2 -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

Table 1. Baseline characteristics of study subjects.

characteristics	anti-TNF group (n = 52)	no anti-TNF group (n = 41)	healthy controls (n = 18)	P-value
Women (%)	71	68	78	ns *
Age, mean (range), years	50 (23 - 73)	47 (18 - 83)	47 (21 - 75)	ns †
rheumatic disease (%)	88	71	-	0.038 ‡
duration of disease activity, mean (months)	149	94	-	0.018 §
prednisone,% (mean dose, mg)	21 (6)	22 (9)	-	ns ‡ ns §
methotrexate,% (mean dose, mg)	65 (17)	76 (16)	-	ns ‡ ns §
azathioprine,% (mean dose, mg)	10 (115)	15 (108)	-	ns ‡ ns §

* χ^2 - 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

‡ χ^2 - 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; NSAIDs: non-steroidal anti-inflammatory drugs; ns: not significant

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%).

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.

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).

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.

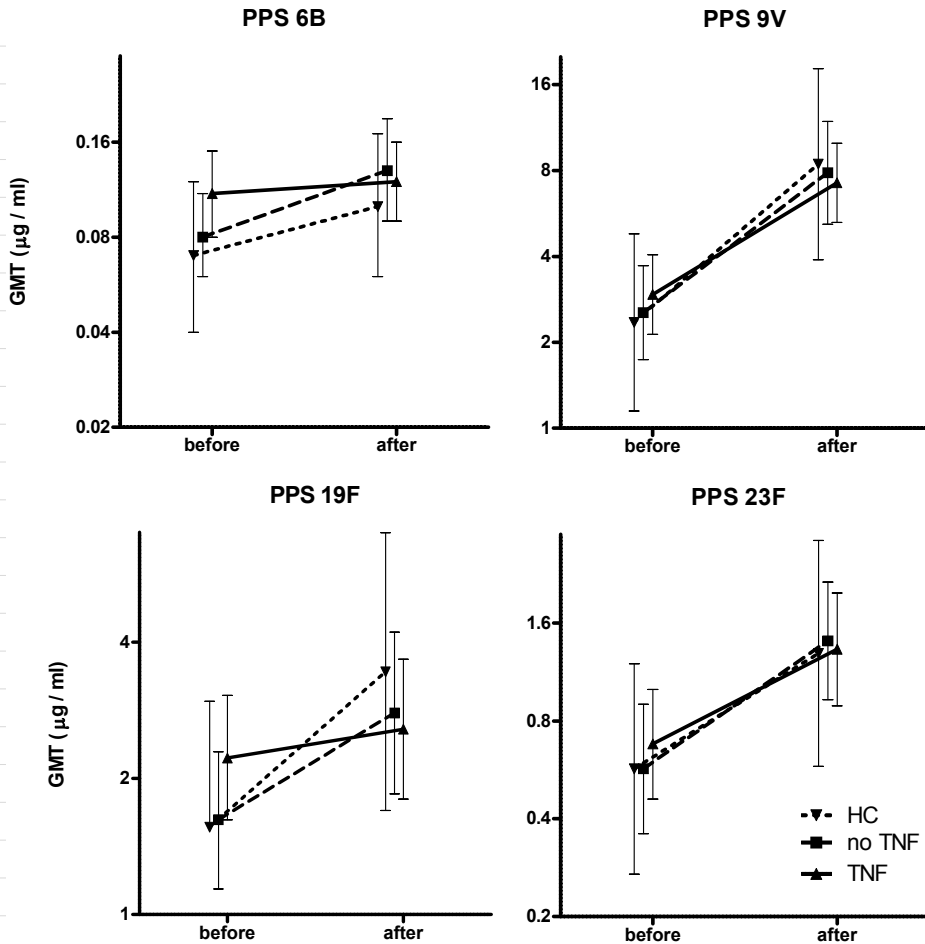


Figure 1. Geometric mean titer (GMT) pre- and postvaccination (error bars indicate 95% confidence interval).

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 $\leq 11\%$, 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).

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

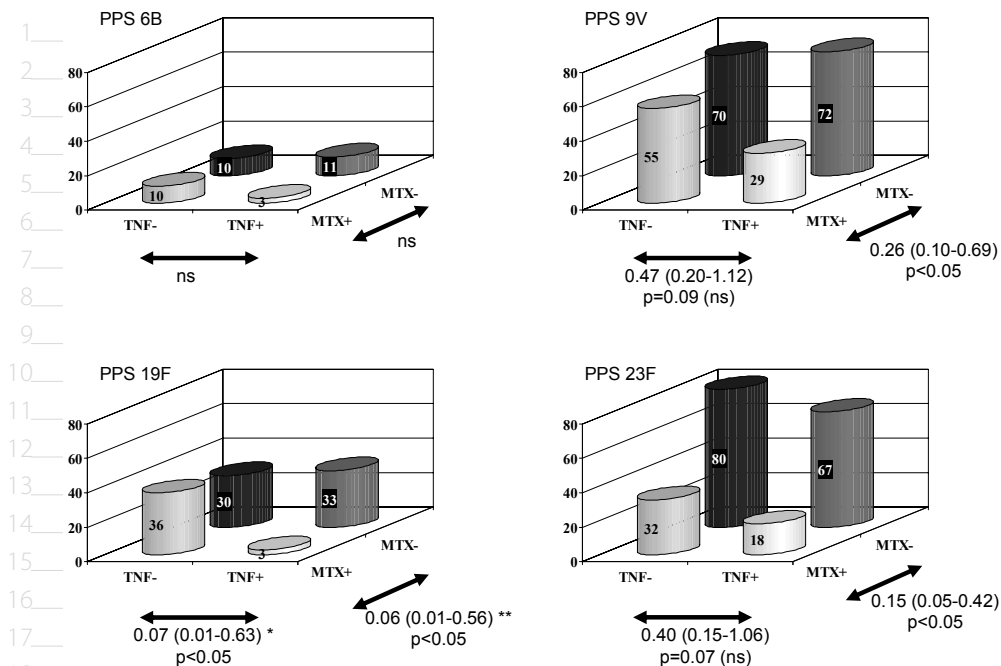


Figure 2. Response rates, defined as the percentage with a postvaccination titer $\geq 0.35 \mu\text{g/ml}$ in combination with at least a two-fold increase in antibody titer to PPS 6B, 9V, 19F and 23F by use of anti-TNF (TNF) and methotrexate (MTX): + denotes use, - denotes no use. Reported are statistically 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.

practice. The general cut off titer of $0.35 \mu\text{g/ml}$ 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

Table 2. The response rate (RR), defined as the percentage with a postvaccination titer ≥ 0.35 $\mu\text{g/ml}$ 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 $\mu\text{g/ml}$, per group.

PPS	anti-TNF group (n = 52)	no anti-TNF group (n = 41)	healthy controls (n = 18)	P-value *
RR 6B	6	10	6	ns
PR 6B	15	24	17	ns
RR 9V	44	59	67	ns
PR 9V	100	100	94	ns
RR 19F	14	34	44	p=0.012
PR 19F	94	93	83	ns
RR 23F	35	44	39	ns
PR 23F	83	83	83	ns

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

* χ^2 -test (2-sided) for anti-TNF versus no anti-TNF versus healthy controls

a severe adverse event, however there were no sequelae. She had a remarkable twenty five-fold 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 $\mu\text{g/ml}$ 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 response 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.

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

9

10 The true clinical efficacy of pneumococcal vaccination (a reduction of invasive disease
 11 and mortality attributable to pneumococcal infections) can only be established in trials
 12 including thousands of participants. Since this is not feasible for patients treated with
 13 anti-TNF, surrogate markers, such as the response rate, are the best estimate of clinical
 14 efficacy. The fact that we found blunted response rates in patients treated with the com-
 15 bination of methotrexate and anti-TNF, should not be used to exclude these patients
 16 from initiatives that promote vaccine uptake in immunocompromised patients. [39-41]
 17 Even in these 'worst responders' we did find antibody titer increases upon vaccination
 18 and high postvaccination titers, which are also correlated with protection from invasive
 19 pneumococcal disease. [27] This study was not designed to evaluate the value of routinely
 20 measuring postvaccination titers in these patients.

21

22 We conclude that the response upon the pneumococcal polysaccharide vaccine is sig-
 23 nificantly impaired in patients treated with methotrexate, especially when methotrexate
 24 is combined with anti-TNF. However, there are no arguments to withhold pneumococcal
 25 vaccination from this subgroup of patients with combined methotrexate and anti-TNF
 26 therapy. Ideally, patients should receive pneumococcal vaccination before immunosup-
 27 pressive therapy with methotrexate and/or anti-TNF is initiated or intensified.

28

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Chapter | 2

The effect of anti-tumor necrosis factor alpha treatment on the antibody response to influenza vaccination

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1 ABSTRACT

2
3 **Objectives.** The effect of anti-TNF therapy on the antibody responses to vaccines is
4 subject of ongoing debate. Therefore, we investigated the effect of the three currently
5 available anti-TNF agents on influenza vaccination outcomes in a patient population with
6 longstanding disease.

7 **Methods.** In a prospective cohort study, we assessed the antibody response upon influenza
8 vaccination in 112 patients with longstanding autoimmune disease treated with immu-
9 nosuppressive medication either with anti-TNF (etanercept, adalimumab or infliximab;
10 n=64) or without anti-TNF (n=48) and a control group of 18 healthy individuals. An-
11 tibody responses were determined by hemagglutination inhibition assay, before and four
12 weeks after vaccination.

13 **Results.** The proportion of individuals with a protective titer (≥ 40) after vaccination was
14 large (80 to 94%) and did not significantly differ between the three groups. Postvaccina-
15 tion geometric mean antibody titers against influenza (A/H3N2 and B) were significantly
16 lower in the 64 patients treated with anti-TNF compared to the 48 patients not receiving
17 anti-TNF and the healthy controls.

18 **Conclusions.** The antibody response to influenza vaccination in patients treated with
19 anti-TNF is only modestly impaired. The proportion of patients that achieves a protective
20 titer is not significantly diminished by the use of TNF blocking therapies.

1 INTRODUCTION

2
3 Anti-tumor necrosis factor alpha treatment (anti-TNF) is effective in autoimmune disorders such as rheumatoid arthritis and Crohn's disease. [1] The three compounds currently registered for clinical use infliximab (Remicade™), etanercept (Enbrel™) and adalimumab (Humira™) differ in the mode of action and incidence rate of opportunistic infections. 7 [2,3]

8 Although data on an increased morbidity and mortality due to influenza in patients treated with anti-TNF are lacking, guidelines recommend that patients at risk for complications of influenza, including those treated with anti-TNF, should be annually vaccinated against influenza. [4-6] Data on the effect of anti-TNF on the response to influenza vaccination are scarce and conflicting. [7-9] The aim of this study is to compare the antibody response upon vaccination against influenza in patients treated with immunosuppressive therapy including anti-TNF, matched patients not treated with anti-TNF, and healthy controls.

18 METHODS

20 Subjects

21 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 the gastro-enterology outpatient clinic. Pregnancy, severe chicken egg allergy or an active infectious disease were exclusion criteria. Approximately 1000 patients with Crohn's disease and 2000 patients with rheumatoid arthritis visit these outpatient clinics yearly; 207 patients (<7%) were treated with anti-TNF. Sixty-nine (33%) of these patients were enrolled of whom 64 completed the two study visits (anti-TNF group). Fifty-four patients selected from the same outpatient clinics who were not treated with anti-TNF and nineteen healthy controls matched for sex and age were also recruited. Respectively 48 (no anti-TNF group) and 18 (healthy controls) also completed both study visits. Side effects or worsening of the underlying condition were not stated as reasons for study discontinuation by any participant. The protocol was designed according to the good clinical research guidelines and approved by the local medical ethical committee; a written informed consent was obtained from all subjects.

36 Vaccine

37 All study subjects were vaccinated in the fall and winter of 2003 with a commercially available trivalent subunit influenza vaccine (Influvac™ 2003/2004, Solvay Pharmaceuticals B.V., Weesp, The Netherlands). The vaccine contained 15 µg of hemagglutinin of each

of the following strains: A/Moscow/10/99 (H3N2) like strain (A/Panama/2007/99 RES-VIR-17 reassortant) further referred to as A/H3N2; A/New Caledonia/20/99 (H1N1) like strain (A/New Caledonia/20/99 IVR-116 reassortant) further referred to as A/H1N1, and B/Hong Kong/330/2001 like strain (B/Shangdong/7/79) further referred to as influenza B. Vaccines were stored at 6°C and administered at room temperature by intra-muscular injection in the left deltoid muscle according to the package insert. Patients registered side effects in a study log.

Antibody assays and statistics

Serum samples were collected at the study visits, at week 0 (before the vaccination) and 4 weeks thereafter and were stored at – 80°C until use. The hemagglutination inhibition (HI) test was performed in duplicate according to standard methods. [10] All sera of each individual study subject were tested simultaneously. For statistical analysis a titer of 5 was arbitrarily assigned to sera with a titer <10. Protection rates (PR) were defined as the percentage of patients with a HI titer ≥ 40 after vaccination, response rates (RR) as a fourfold titer increase, in accordance with European guidelines. [11] The results were analyzed by standard statistical methods: One-Way ANOVA for geometric mean titers (GMTs), two-sided χ^2 -test for PR and RR. Calculations were performed using SPSS, version 14.0.

RESULTS

One hundred and twelve patients (71% female, median age 49 years, range 18 - 85) and 18 healthy controls (78% female, median age 52 years, range 21 - 75) were evaluated (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) and non-steroidal anti-inflammatory drugs (NSAIDs). The percentage of patients with rheumatic diseases and the percentage of patients who received prior influenza vaccination, in at least one of the three years before this study, were significantly higher in the anti-TNF group compared to the control patients. Within the anti-TNF group all three currently available compounds were used, on average for 24 months (range 0.5 to 78 months): infliximab by 29 (45%), including all IBD patients, etanercept by 14 (22%) and adalimumab by 21 (33%).

Four weeks after vaccination against influenza the anti-TNF group had lower GMTs when compared to both the no anti-TNF group and healthy controls. This was significant for two of the three vaccine components (A/H3N2 and influenza B). The no anti-TNF group had insignificantly lower GMTs after vaccination than healthy controls (figure 1). Response rates were significantly lower in the anti-TNF group compared to the no anti-TNF group ($p < 0.05$ for all three antigens). Using slightly different outcome measurements, as used by other authors, did not change our results. [7-9]

Table 1. Baseline characteristics of study subjects.

Characteristics	Anti-TNF group (n = 64)	No anti-TNF group (n = 48)	Healthy controls (n = 18)	P-value
Women (%)	73	69	78	0.74 ^a
Age, mean (range), y	51 (23 – 75)	46 (18 – 85)	47 (21 – 75)	0.23 ^b
GE disease (%)	9	33	-	0.002 ^c
RA (n)	52	27	-	
JCA (n)	2	2	-	
M. Still (n)	2	0	-	
PA (n)	1	3	-	
SA (n)	1	0	-	
M. Crohn (n)	6	15	-	
IBD (n)	0	1	-	
Duration of disease activity, mean (months)	156	111	-	0.041 ^d
Prednisone (%)	20	23	-	0.74 ^c
Mean daily dose, mg	7	9	-	0.58 ^d
Methotrexate (%)	71	75	-	0.58 ^c
Mean weekly dose, mg	16	15	-	0.72 ^d
Azathioprine (%)	8	17	-	0.15 ^c
Mean daily dose, mg	115	109	-	0.73 ^d
NSAIDs (%)	57	58	-	0.90 ^c
Prior vaccination,%	62	38	6	< 0.001 ^a

^a χ^2 -square test (2-sided) for anti-TNF versus no anti-TNF versus healthy controls

^b One-way ANOVA for anti-TNF versus no anti-TNF versus healthy controls

^c χ^2 -square test (2-sided) for anti-TNF versus no anti-TNF

^d Wilcoxon rank sum test (2-sided) for anti-TNF versus no anti-TNF

TNF: tumor necrosis factor alpha; GE: gastro-enterologic; RA: rheumatoid arthritis; JCA: juvenile chronic arthritis; PA: psoriatic arthritis; SA: spondyloarthropathy; IBD: inflammatory bowel disease; NSAIDs: non-steroidal anti-inflammatory drugs

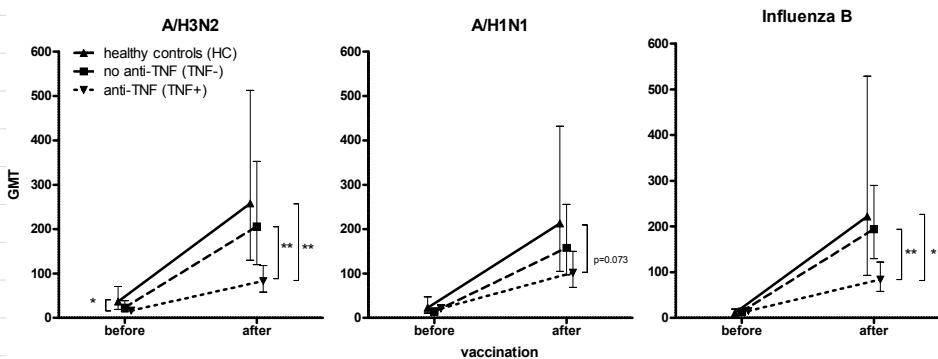


Figure 1. Crude geometric mean titers (error bars indicate 95% confidence interval) at week 0 and 4 for respectively influenza A/H3N2, A/H1N1 and influenza B.

anti-TNF: anti-tumor necrosis factor alpha therapy.

* $p < 0.05$; ** $p < 0.005$

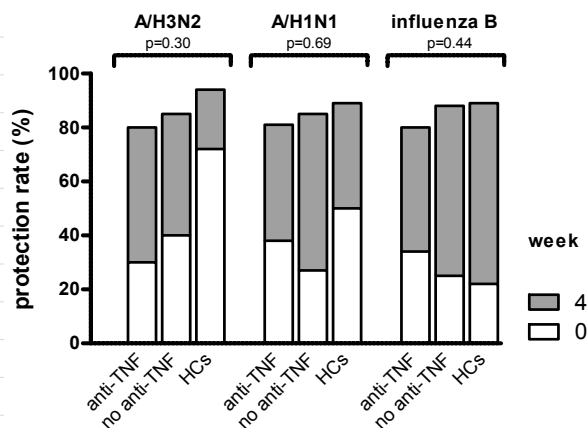


Figure 2. Protection rates before (pre) and 4 weeks after (post) vaccination. P-values from two-sided Pearson's χ^2 -test between groups.

Protection rates four weeks after vaccination were high in all groups tested (figure 2). The percentage with protective titers was 89 – 94% at week 4 in healthy subjects. In both patient groups these percentages were slightly lower, with no statistically significant differences between the three groups.

Infliximab, etanercept and adalimumab, when tested separately, exert similar effects on the response to vaccination. Methotrexate, prednisone, azathioprine and NSAIDs did not influence post-vaccination titers significantly when compared to control groups (data not shown). Linear regression analysis on post-vaccination titers with age, gender, underlying disease, rheumatoid factor positivity, history of previous influenza vaccinations, and prevaccination titers as independent variables, showed that only prevaccination antibody titers and a history of previous influenza vaccinations significantly contributed to variations in postvaccination titers. Prevaccination titers in healthy controls, resulting from natural infection, were higher compared to both patient groups. After correction the anti-TNF group still had lower postvaccination titers than the no anti-TNF group.

No major side effects and no deterioration of the underlying condition were attributed to influenza vaccination. Minor side effects, such as local pain or tenderness, were frequently reported (20%), with no differences between the three groups. Systemic reactions like fever, myalgia or headache were reported in 14% of all patients during the 4 weeks after vaccination.

DISCUSSION

The present study demonstrates adequate protection rates against influenza after influenza vaccination in patients treated with anti-TNF, despite lower postvaccination antibody titers

and lower response rates as compared to both similar patients not treated with anti-TNF and healthy controls. To our knowledge this is the first study to include both patients with gastro-enterologic and rheumatologic diseases with longstanding auto-immune diseases requiring treatment, mirroring daily practice. In the present study all three commercially available anti-TNF compounds were used and the average time on this therapy was almost two years. In a multivariate analysis, underlying disease (either rheumatologic or IBD), the use of methotrexate or the type of anti-TNF compound used (either infliximab, etanercept or adalimumab) did not influence vaccination outcomes. Up to now, only a few studies have reported on the antibody response upon influenza vaccination in patients treated with anti-TNF. [7-9] In line with the current study, these studies agree upon the clinically relevant finding that treatment with anti-TNF does not lower the protection rate (table 2).

The seemingly paradoxical finding of significantly lower postvaccination titers (and response rates), but equal protection rates, is explained by the fact that the protection threshold (a titer ≥ 40) is relatively easily met. Actual postvaccination titers are therefore considered a better parameter reflecting the immunological competence of a group. This modest, but immunologically relevant, effect was similar for all three antigens and was also consistent in various subgroup analyses (RA vs. IBD patients; patients seronegative before vaccination) although power was lacking to draw firm conclusions concerning these subgroups.

Two other studies report pre- and post vaccination titers after influenza vaccination in patient groups treated with and without anti-TNF. [7,8] In both studies postvaccination titers were lower in the group treated with anti-TNF as compared to a control group for all three influenza antigens. In the study by Kaine *et al.* patients received only a single dose of adalimumab before vaccination, which might explain the relative modest effect of anti-TNF on GMTs in this study (table 2). [7]

Inhibition of TNF, a pivotal cytokine in the type-1 (cell-mediated) immune responses, is most noticeable in T-cell mediated reactions. We therefore expected a greater effect of anti-TNF on the T-cell-dependent responses to the influenza subunit vaccine as compared to the effect on T-cell-independent responses. In most vaccination studies conducted in patients treated with anti-TNF, the T-cell-independent pneumococcal polysaccharide vaccine was used and, as expected, none of these studies reported a negative impact of anti-TNF on vaccination outcomes. [12-15]

In a population consisting of patients with longstanding autoimmune diseases, receiving immune modulating treatment, the proportion of patients with protective titers after a single vaccine dose is substantial, about 80%, irrespective of the immunosuppressive medication or underlying condition. This endorses the current guidelines for a single, annual influenza vaccination of these patients, including those treated with anti-TNF.

Table 2. Comparison of study outcomes of the publications reporting influenza vaccination results in patients treated with anti-TNF. The study outcomes are expressed as percentage higher (+) or lower (-) postvaccination outcomes in the different study groups as compared to a reference patient group.

study	population	n	anti-TNF type (n)	duration anti-TNF	% mtx	mean age, y	GMTs (*1)	protection rates (*2)
Gelinck (present study)	Mixed	64	i/e/a (29/14/21)	2 y	65	51	-60 / -35 / -57	-6 / -5 / -9
		48	-	-	76	47	reference group	
	HC	18	-	-	-	47	+25 / +35 / +14	+11 / +5 / +1
author conclusions	'significant inhibition of GMTs by anti-TNF, without lowering protection rates'							
Kaine 2007 [7]	RA	99	a	1 gift (40mg)	56	52	-22 / -14 / -20	-19 (t)
		109	-	-	54	51	reference group	
author conclusions	'adalimumab does not diminish humoral response, the majority of patients are protected'							
Kapetanovic 2006 [8]	RA	50	i/e (37/13)	0.7 y	100	53	-42 / -70 / -49	-26 / -42 / -12
		62	i/e (27/35)	1.1 y	0	54	+12 / -60 / -39	-3 / -35 / -8
		37	-	-	100	61	reference group	
	HC	18	-	-	-	30 (?)	-15 / -31 / -61	-5 / -12 / -29
author conclusions	'immune response sufficiently large, regardless of treatment'							
Fomin 2006 [9]	RA	27	i/e (22/5)	>= 3 m	68	59	not reported seperately	+31 / +27 / +18 (#1)
		55	-	-			reference group	
	HC	30	-	-	-	53	+9 / +9 / +15	+2 / +28 / +30 (#2)
author conclusions	'infliximab does not affect the humoral response'							

(*1) relative percentage higher or lower postvaccination GMTs as compared to reference patient group (for respectively influenza A/H3N2, A/H1N1 and influenza B); '-70' indicates a postvaccination titer 70% lower than the reference group.

(*2) relative percentage higher or lower protection rate as compared to reference patient group (for respectively influenza A/H3N2, A/H1N1 and influenza B), unless stated otherwise; '+10' indicates a (relative) 10% higher protection rate as compared to the reference group.

(+) pooled data of 3 antigens (protective antibodies against >= 2 out of 3 influenza antigens)

(?) based on mean age of 47 healthy controls in pneumococcal vaccination study

(#1) difference (%) in percentage of responders (defined as either seroconversion or a fourfold titer increase in patients with a protective titer before vaccination) in patients treated with infliximab (n=22) vs. no infliximab (n=60, including 5 patients with etanercept)

(#2) difference (%) in percentage of responders (defined as either seroconversion or a fourfold titer increase in patients with a protective titer before vaccination) in all patients (n=82) vs. healthy controls (n=30)

anti-TNF: anti-tumor necrosis factor alpha therapy; mtx: methotrexate; GMT: geometric mean titer

RA: rheumatoid arthritis; HC: healthy control; i: infliximab; a: adalimumab; e: etanercept;

y: years; m: months

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Appendix | 1

Appendix to chapter 1 and 2 Immune Response in Immunocompromised Hosts (RICH)-1 study – additional data

Unpublished supplemental data

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Immune Response in Immunocompromised Hosts (RICH)-1 study – additional data

Study scheme

The pneumococcal (chapter 1) and influenza (chapter 2) vaccination (RICH-1) studies were done simultaneously within the same study population. The study scheme is represented in Figure 1: patients received an influenza vaccination at week 0, with a second influenza vaccination at week four, to study if this would increase the number of subjects with a protective titer. At week 4 a pneumococcal vaccination was administered simultaneously with the influenza vaccination, a final evaluation was done at week 8.

subjects (n)		*	**
healthy controls	18	18	18
no anti-TNF	48	48	41
anti-TNF	64	64	52

vaccinations	↓	↓↓	
weeks	0	4	8

Figure 1. Flowchart with a schematic representation of the RICH-1 study, including the number of participants at the different time points.

The number of patients lost-to-follow up was high in this study, increasing with every study visit. An important reason for this was that anti-TNF was not readily available in 2003. Anti-TNF was used in clinical trials as a rescue treatment for patients failing other drug regimens. Many patients travelled long distance to be included in these treatment trials, the travel associated with the extra visits for the vaccination study proved to be problematic for some of the patients.

Follow up

that completed all three study visits, revaccination did not give rise to a booster response (Figure 2); only a modest increase of patients with a protective titer was seen (Figure 3). Thus, repeated influenza vaccination with only a four week interval does not provide a relevant increase of anti-influenza. The influenza follow-up data are presented in Figure 2 and 3. Within the group of patients immunity.

Evaluation of different immunological pathways

As described in the general discussion of this thesis, evaluating both a T-cell-dependent (influenza) and T-cell-independent (pneumococcal) vaccine, within the same patient cohort gave us the opportunity to study the immunological properties of anti-TNF alone and in combination with other drugs. Table 1 shows correlations between the response on influenza antigens (the protection rate as defined in chapter 2) and the response upon

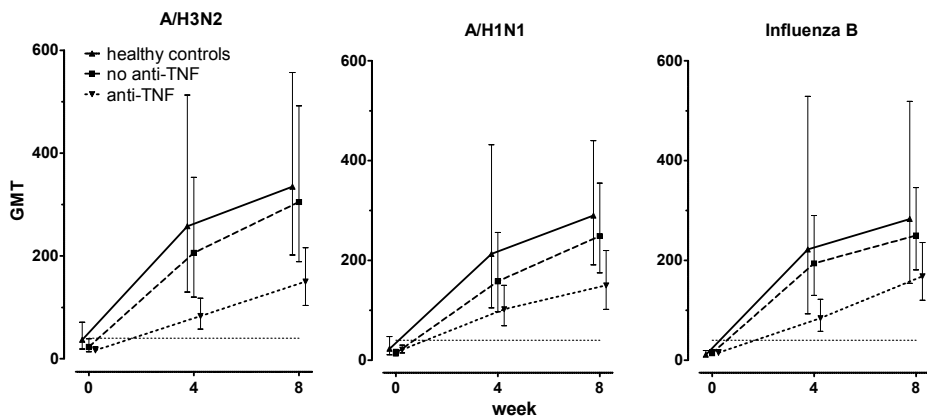


Figure 2. Geometric mean titers (95% confidence interval) against influenza A/H3N2, A/H1N1 and influenza B at the different time points.

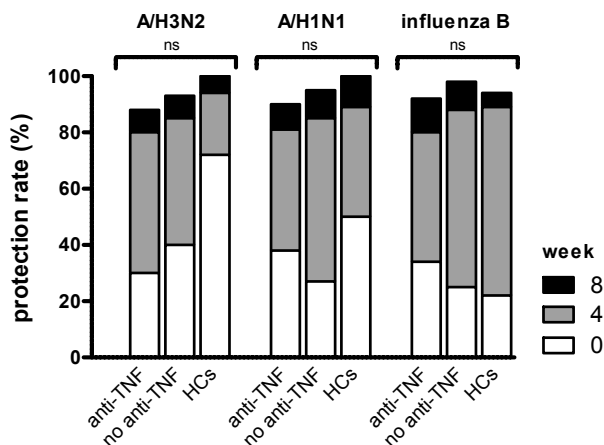


Figure 3. Protection rates (the percentage of subjects with a titer >40) at week 0 (prevaccination), 4 and 8 for influenza A/H3N2, A/H1N1 and influenza B.

pneumococcal antigens (the response rate as defined in chapter 1). The strength of the correlation is coded by the colour of the cells, p-values are coded by asterixes. As would be expected, there are clear correlations among the influenza antigens on one hand and pneumococcal antigens on the other hand. There is, however, a striking absence of correlations between the response upon influenza and pneumococcal antigens. Most correlations found were weak (Pearson's correlation coefficient <0.5), only responses upon the two influenza A strains (H3N2 and H1N1) and the responses upon PPS 19F and two of the other PPSs showed stronger correlations. The fact that the responders upon influenza vaccination and the responders upon pneumococcal vaccination do not correspond with each other, underscores the fact that different immunological pathways are being evaluated.

Table 1. Cross tabulation, indicating positive correlations for responders upon influenza and PPS antigens.

		H3	H1	B	6B	9V	19F	23F
A/H3N2	HC		**					
	TNF-		*	**				
	TNF+		*					
A/H1N1	HC	**						
	TNF-	*		**				
	TNF+	*						
B	HC							
	TNF-	**	**					
	TNF+							
PPS6B	HC							
	TNF-						**	*
	TNF+							
PPS9V	HC							
	TNF-							*
	TNF+					**	**	**
PPS19F	HC							
	TNF-				**			**
	TNF+					**		*
PPS23F	HC							
	TNF-				*	*	**	
	TNF+					**	*	

Pearson's correlation coefficient: white < 0.3; grey 0.3-0.5; black > 0.5

* correlation is significant at 0.05 level (two-tailed); ** significant at 0.01 level (two-tailed)

In conclusion repeated influenza vaccination with only a four week interval does not provide a relevant increase of anti-influenza immunity. Influenza and pneumococcal vaccination respectively evaluate T-cell-dependent and T-cell-independent pathways.

Chapter | 3

Poor serological responses upon influenza vaccination in patients with rheumatoid arthritis treated with rituximab

Ann Rheum Dis 2007;66:1402-3.

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1__ ABSTRACT

2__
3__ **Objectives.** We examined the impact of rituximab on influenza vaccination outcomes in
4__ rheumatoid arthritis (RA) patients.

5__ **Methods.** Four RA patients treated with Rituximab, 25 RA patients treated with anti-tu-
6__ mor necrosis factor alpha (TNF), and 20 healthy controls, were vaccinated with a trivalent
7__ influenza vaccine. Hemagglutination inhibition (HI) titers were measured to determine
8__ the response upon vaccination.

9__ **Results.** Significantly lower postvaccination titers were found in the RA patients treated
10__ with rituximab, compared to both control groups.

11__ **Conclusions.** Rituximab impairs the ability to respond adequately to influenza vaccina-
12__ tion.

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1 INTRODUCTION

2
3 Annual influenza vaccination is recommended for immunocompromised patients at the
4 beginning of each winter season, under the assumption that it will provide protection
5 against the circulating viral strains during the influenza season. [1,2] Rituximab, an anti-
6 CD20 monoclonal antibody which was recently approved by American and European
7 authorities for the treatment of RA patients failing anti-TNF, diminishes circulating
8 B-cells for a period of 6 to 9 months after infusion. [3-5] The package insert states that
9 patients should not be vaccinated from 1 month before administration of rituximab until
10 6 months thereafter based on data in lymphoma patients. [6-8] In these studies rituximab
11 influenced primary as well as secondary immune responses to neoantigens (keyhole limpet
12 hemocyanin and hepatitis A vaccine) and recall antigens (tetanus toxoid and poliomyelitis
13 vaccine). [7] Certainly the underlying lymphoma and treatment with chemotherapy
14 contributed to the diminished immunological responses in these patients. The effect of
15 rituximab on influenza vaccination outcomes in RA, therefore, is not known.

18 METHODS

19
20 We examined the humoral responses upon influenza vaccination in four RA patients (3/4
21 female, age range 55-61) all treated with rituximab combined with methotrexate (5-20
22 mg weekly) and prednisone (5 mg) in one patient and compared these to responses of 25
23 patients treated with anti-TNF with or without disease modifying anti-rheumatic drugs
24 (DMARDs; 64% female, mean age 54 years, range 37-71), and 20 healthy controls (50%
25 female, mean age 45 years, range 19-77). The four patients received 1000 mg rituximab
26 intravenously on days 0 and 14. Infusions were combined with 100 mg prednisolone and
27 2 mg clemastine. A timeline summarizing these events, as well as the waning of B-cell
28 counts after rituximab infusion in the four individual patients, is presented in figure 1.

29 Patients were vaccinated intramuscularly, as part of a larger study, with a trivalent
30 subunit vaccine (Influvac 2005-2006, Solvay, Weesp, The Netherlands; 0.5 mL contain-
31 ing 15 µg hemagglutinin of each of the following strains: A/California/7/04 like virus
32 (further referred to as A/H3N2), A/New Caledonia/20/99 like virus (further referred to
33 as A/H1N1) and B/Shanghai/361/02 like virus (further referred to as influenza B). The
34 influenza vaccine was administered between 87 – 140 days after the first rituximab infu-
35 sion (figure 1). HI titers were measured (in duplo) just before vaccination and 28 days
36 later as described before. [9,10] We report (geometric) mean titers (GMTs) as our main
37 outcome to compare groups. [11] Protection rates (the percentage of a group with a titer ≥
38 40, which is considered to be protective) were calculated. For B-cell counts blood samples
39 were obtained at baseline and at days 28, 84 and 168 after starting rituximab treatment.

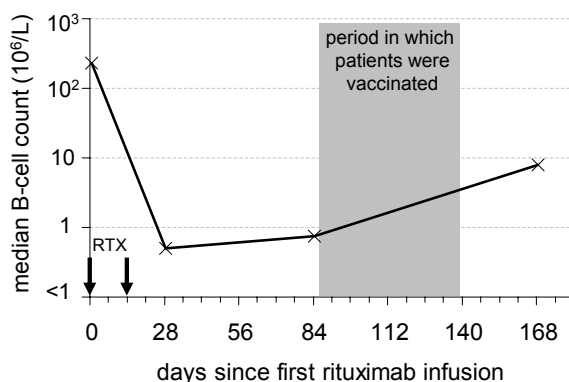


Figure 1. Graphical representation of rituximab (RTX) infusions, the period in which the influenza vaccine was administered and the median B-cell count in time.

B-lymphocytes were identified using a 4-color stain-lyse-no wash platform method, using incubation with a mixture of monoclonal antibodies against CD45 (clone 2d-1), CD3 (clone SK-7) and CD19 (clone 5J25C1)(BD biosciences, San Jose, CA, USA). Absolute lymphocyte counts were analyzed by TruCOUNT tubes using flowcytometry. The lower limit of detection for B-cells was 1×10^6 cells per L. Pearson's chi-square test and ANOVA statistics were used where applicable.

RESULTS

The 4 RA patients treated with rituximab had significantly lower postvaccination titers compared to both control groups (RA patients treated with anti-TNF with or without DMARDs and healthy controls), as shown in figure 2. Postvaccination protection rates

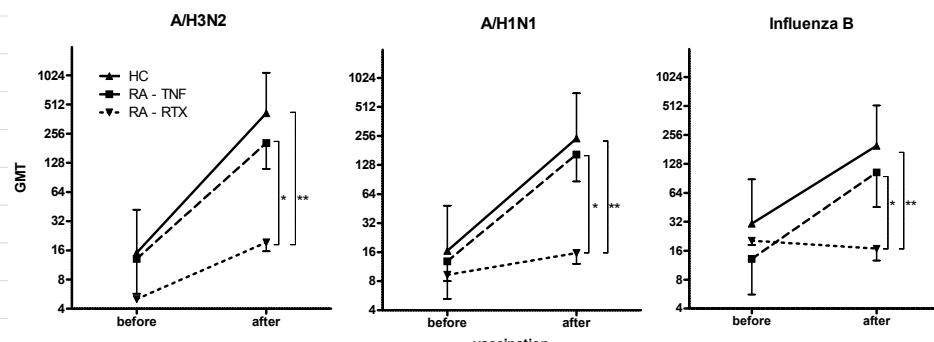


Figure 2. Pre- and postvaccination serum geometric mean titers (GMT with standard deviation) against influenza A/H3N2, A/H1N1 and Influenza B for a group of RA patients treated with rituximab (RA - RTX), RA patients treated with anti-TNF (RA - TNF) and healthy controls (HC). * $p=0.02$; ** $p < 0.001$.

also were significantly lower in the rituximab group (25-50%) compared to both control groups (84% for all three antigens in the RA group and 85-100% in healthy controls, $p < 0.05$ for all three antigens with Pearson's chi-square test). The three groups were well matched with regard to sex, age, prevaccination titers and prior influenza vaccination. B-cells were completely depleted in all four patients at day 84 after the first rituximab infusion. Only one patient had complete B-cell recovery at day 168. This patient was vaccinated 3 days after the nadir measurement (day 84: $1 \times 10^6/L$). No major side effects were observed after vaccination nor any effect on disease activity.

DISCUSSION

Even though only four RA patients treated with rituximab were evaluated, we found significantly lower postvaccination titers (figure 1) and protection rates (the proportion of a group with a titer ≥ 40) in comparison to compared to a group of RA patients treated with anti-TNF and a group of healthy controls for all three antigens. The influenza vaccine was administered 3 to 5 months after the first rituximab infusion at the time of early B cell recovery ($<10 \times 10^6$ CD19⁺ cells/L in blood). In keeping with previous studies, influenza vaccination was effective in RA patients treated with anti-TNF, although the induced concentrations of antibodies against the three antigens were slightly lower than in healthy controls. [12,13] Thus, the impaired effectiveness in rituximab treated RA patients cannot be explained by the presence of underlying disease or the use of other immunosuppressive medication. One other study reported a significantly lower response rate for only one out of three antigens in RA patients treated with rituximab. [14] The comparability to our results is limited since responses were poor in all groups and no information was provided on rituximab dose and number of B-cells at time of vaccination.

In conclusion, our findings suggest that influenza vaccination, although not completely ineffective, will most likely not sufficiently protect rituximab treated RA patients from influenza infection during the whole winter season. Other preventive strategies and precautions, such as vaccinating household members and pre-emptive or early treatment with anti-viral medication after exposure, should also be considered.

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Chapter | 4

Intradermal influenza vaccination in immunocompromised patients is immunogenic and feasible

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1 ABSTRACT

2
3 **Background.** Many strategies, including intradermal vaccination, have been tested to
4 augment antibody responses upon vaccination. This strategy has not been evaluated in dif-
5 ferent groups of immunocompromised patients. We conducted a prospective, randomized
6 study to compare the humoral response upon standard intramuscular influenza vaccina-
7 tion with the response upon reduced-dose intradermal vaccination in patients treated with
8 anti-tumor necrosis factor (TNF)-alpha, human immunodeficiency virus (HIV)-infected
9 patients, hematologic stem cell transplantation (HSCT) patients, and healthy controls.

10 **Methods.** In total 156 immunocompromised patients and 41 healthy controls were
11 randomized to receive either 0.5 mL of the 2005/2006 trivalent influenza vaccine intra-
12 muscular or 0.1 mL intradermal. Humoral responses, determined by hemagglutination
13 inhibition assay, were measured before and 28 days post vaccination. Geometric mean
14 titers (GMTs) and protection rates (PRs) are reported as primary outcomes, adverse events
15 as a secondary outcome.

16 **Results.** Reduced-dose intradermal vaccination leads to similar GMTs and PRs, within
17 all tested groups, compared to the standard intramuscular vaccination. Healthy controls
18 yielded significantly better GMTs and PRs than immunocompromised patients. Local
19 skin reactions after intradermal vaccination occurred less frequent and were milder in
20 immunocompromised patients than in healthy subjects and were predictive for a positive
21 vaccination outcome for individual subjects.

22 **Conclusions.** Intradermal influenza vaccination is a feasible alternative for standard
23 intramuscular vaccination in several groups of immunocompromised patients, including
24 those treated with anti-TNF, HIV-infected patients and HSCT patients. The occurrence
25 of a local skin reaction after intradermal vaccination is predictive of a response to at least
26 one of the vaccine antigens.

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1 BACKGROUND

2

3 Annual influenza vaccination is recommended for immunocompromised patients. [1-4]
 4 Besides causing a greater risk of complications from influenza infection, an immunode-
 5 ficiency compromises the response to (T-cell-dependent) vaccine antigens. [5-7] Many
 6 strategies have been explored in immunocompromised patients in order to optimize
 7 vaccination outcomes, including increased dosage, multiple dose vaccination, the use of
 8 vaccine adjuvant, immunostimulant patches and more efficient routes of vaccine delivery.
 9 [8-12]

10 In persons with impaired immunity intradermal vaccination is of particular interest
 11 because of anticipated immunologic advantages. The dermis harbours a network of an-
 12 tigen presenting cells, constituting up to two percent of all dermal cells, which forms an
 13 optimal environment to deliver a vaccine. [13-15] Since there is a dose-response relation
 14 for antigen quantity and antibody response, studies in immunocompromised patients have
 15 mostly concentrated on higher vaccine doses administered intramuscularly. [6, 16] The
 16 favourable immunologic properties of the dermis allow for smaller quantities of vaccine
 17 to be used. Healthy subjects respond to reduced-dose intradermal influenza vaccination
 18 generally just as well as to standard intramuscular vaccination, especially in populations
 19 already primed with the antigen. [17-26] Intradermal influenza vaccination received most
 20 attention in times of vaccine shortage caused by pandemics (e.g. the 1957 influenza A/
 21 H2N2 pandemic) or manufacturing problems as occurred in 1973 and 2004. [27, 28]
 22 These studies concentrated on the responses of healthy subjects. Only little information
 23 is available on intradermal influenza vaccination in immunocompromised patients. [29]

24 Besides a potential immunologic benefit, there is an obvious economic advantage in
 25 saving up to 80% of the vaccine required, allowing for full vaccination coverage even
 26 in times of vaccine shortages. [30-31] There are however also some inherent practical
 27 disadvantages to intradermal vaccination, especially relevant in mass vaccination cam-
 28 paigns such as the annual influenza vaccination campaigns or the response to an eventual
 29 pandemic influenza outbreak. The vaccination technique itself is more difficult and time
 30 consuming than intramuscular vaccination. Furthermore, local side effects in healthy
 31 subjects, are more severe and frequent upon intradermal vaccination when compared to
 32 intramuscular vaccination, a factor known to negatively impact vaccine uptake. [32-33]
 33 These factors and the small number of data on intradermal vaccination in subjects with an
 34 indication for annual influenza vaccination have likely contributed to the decision from
 35 the Center of Disease Control and Prevention (CDC) to discourage intradermal influenza
 36 vaccination in its 2007 guidelines, especially for persons older than sixty years. [1]

37 Before intradermal influenza vaccination can be implemented as a routine procedure,
 38 the efficacy and safety should be established in patient groups with an indication for
 39 annual influenza vaccination. We conducted a prospective, randomized study to test the

hypothesis whether reduced-dose intradermal influenza vaccination in immunocompromised patients, in particular those treated with anti-tumor necrosis factor (TNF) alpha, persons infected with the humane immunodeficiency virus (HIV) and patients who have undergone hematological stem cell transplantation (HSCT), is safe and whether it leads to an equal quantitative serologic response as compared to the response upon standard intramuscular vaccination.

METHODS

Trial design and subjects

A group of healthy controls (*HC group*) and three groups of immunocompromised patients were included in this trial. Rheumatologic patients treated with anti-TNF with or without other immunosuppressives at the Leiden University Medical Center (LUMC), Leiden or the Sint Maartenskliniek, Nijmegen, The Netherlands (*anti-TNF group*); HIV infected patients in care at the LUMC (*HIV group*) and HSCT patients treated at the LUMC (*HSCT group*) were asked to participate.

Importantly, this study was designed to mirror clinical practice, with few limitations for inclusion. Exclusion criteria included only known allergy to influenza vaccine, severe chicken egg allergy, age below 18 years, the use of oral anticoagulation therapy, a thrombocyte count less than 25×10^9 per liter and a skin type not suitable for intradermal vaccine delivery (such as severe corticosteroid-induced skin atrophy). The four study groups were randomized to receive either standard intramuscular vaccination or reduced dose intradermal vaccination. Permuted-block randomization was performed with the use of sealed envelopes containing the balanced vaccination codes for ten subjects. All subjects were asked to register adverse reactions in a study log. The study protocol was approved by the appropriate institutional ethics committees (LUMC, local CME number P05.115; Sint Maartenskliniek local CME number RR-14-GRIEP; ISRCTN15762138) and conducted in accordance with de Declaration of Helsinki. All participants provided a written informed consent.

Vaccine and vaccination

All study subjects were vaccinated in the fall and winter of 2005 with a commercially available trivalent subunit influenza vaccine (Influvac™ 2005/2006, Solvay Pharmaceuticals B.V., Weesp, The Netherlands) at day 0. The vaccine contained 15 µg of hemagglutinin of each of the following strains: A/California/7/04 (H3N2) like strain (A/New York/55/2004 NYMC X-157 reassortant) further referred to as A/H3N2; A/New Caledonia/20/99 (H1N1) like strain (A/New Caledonia/20/99 IVR-116 reassortant) further referred to as A/H1N1, and B/Shanghai/361/02 like strain (B/Jiangsu/10/03) further referred to as

1 influenza B. The A/H3N2 was the only new strain in this vaccine: the A/H1N1 vaccine
 2 strain remained unchanged since the season of 2000/2001; the influenza B strain was
 3 identical to the strain used in 2004/2005.

4 Vaccines were stored at 6°C and administered at room temperature. Intramuscular
 5 vaccinations (0.5 mL) were given according to the package insert by injection in the left
 6 deltoid muscle. On a daily base vaccine for the intradermal administration was transferred
 7 from the pre-filled vaccination syringe into sterile vials and 0.1 mL of vaccine fluid,
 8 containing 3 µg of hemagglutinin of the three strains, was then delivered intradermal in
 9 the palmar side of the forearm according to the CDC guidelines using an intradermal
 10 injection syringe (BD Micro-Fine 0.5 mL U-100 insulin syringe) and needle (29G), also
 11 used for intradermal tuberculin injections. [34] All vaccines were administered by the
 12 investigators who were trained in both vaccination techniques. Directly after vaccination
 13 wheals were measured to confirm adequate intradermal vaccine delivery.

14

15 Antibody assays and statistics

16 Serum samples were collected at day 0 (before vaccination) and day 28 and were stored at
 17 -80°C until use. The hemagglutination inhibition (HI) test was performed in duplicate
 18 according to standard methods with turkey erythrocytes and four hemagglutinating units
 19 of virus to measure antibodies against each of the three vaccine strains as described before.
 20 [5] Ferret sera raised against the test antigens were used as positive controls. All sera of
 21 each individual study subject were tested simultaneously. For statistical analysis a titer of
 22 5 was arbitrarily assigned to sera with a titer <10. Titers were transformed to a logarithmic
 23 scale and geometric means were used for further calculations. Geometric mean titers are
 24 the strongest markers of the immunological capability of a group to respond to an antigen
 25 and were therefore preferred over the response rate (fourfold titer increase), or other out-
 26 come measurements that depend heavily on prevaccination titers. Protection rates (PR)
 27 were defined as the percentage of patients with a HI titer ≥ 40 , which is considered to be a
 28 clinically relevant titer, known to be associated with protection against severe influenza in
 29 healthy controls, after vaccination. [5, 35] The results were analyzed by one-way ANOVA
 30 for geometric mean titers (GMTs), two-sided χ^2 -test for PR.

31 Multivariate analysis of postvaccination GMTs, using a logistic regression model in-
 32 cluding the variables age, sex, previous vaccination, prevaccination titer and vaccination
 33 route was performed on the four different study groups. Group specific variables were
 34 added to the model were applicable (DAS28, type of anti-TNF, use of methotrexate in
 35 the anti-TNF group; a CD4-count of less than 200 cells per mm³, CD4 nadir, the use of
 36 HAART in the HIV group; type of transplantation and being within the first year post
 37 transplantation in the HSCT group).

38 A p-value <0.05 was considered to indicate a statistical significant difference between
 39 groups. Calculations were performed using SPSS for Windows, version 14.0.

1 **RESULTS**

3 **Baseline characteristics**

4 Baseline characteristics of the 197 participants who were randomized are summarized in
5 Table 1. The 41 healthy controls included many healthy partners of patients and hospital
6 staff. The hospital endorses an active policy of influenza vaccination, explaining the relatively
7 high percentage that received a prior influenza vaccination. Fifty patients treated with anti-
8 TNF (fourty RA patients, six with spondyloarthropathy, three with psoriatic arthritis and
9 one with juvenile chronic arthritis) were well matched with regard to gender, age, percentage
10 of patients with RA, disease activity score (DAS28), type of anti-TNF used (two-third were
11 treated with etanercept, one sixth each with infliximab and adalimumab) and other im-
12 munosuppressives (methotrexate was used by 28% of the patients and prednisone by 12%).
13 The percentage of study subjects in the anti-TNF group vaccinated in previous influenza
14 seasons was almost twice as high in the intradermal group as compared to the intramuscular
15 group (p=0.08). A DAS28 of 3.7 represents a moderately severe disease activity.

16 Eighty HIV infected patients were well matched with regard to gender, age, prior
17 influenza vaccinations, actual CD4 count and time on antiretroviral therapy. Over 80%
18 of patients were treated with antiretroviral therapy, the mean time on therapy exceeded
19 five years. The mean CD4 nadir was 166 and 193 cells/mm3 in the intramuscular and
20 intradermal arm respectively. Only 10 patients with a CD4 count of <200 cells / mm3
21 were included.

23 **Table 1.** Baseline characteristics of subjects.

characteristics	HCs		anti-TNF		HIV		HSCT		p-value
vaccination route	im	id	im	id	im	id	im	id	
n	20	21	25	25	41	39	13	13	
male,%	50	48	36	36	71	80	54	39	ns
age, mean, years (range)	45 (19-77)	42 (20-60)	54 (37-71)	53 (36-81)	46 (22-75)	46 (20-60)	55 (40-63)	50 (28-60)	ns
previously vaccinated,%	25	48	28	52	61	59	46*	46*	ns
DAS28, mean	-	-	4.0	3.1	-	-	-	-	ns
CD4, mean, cells/mm3 (range)	-	-	-	-	440 (65- 840)	486 (74- 1356)	-	-	ns
time since hsct , months (range)	-	-	-	-	-	-	19 (4-56)	21 (1-61)	ns

36 * percentage denotes vaccination status of subjects, not of donors (in case of allograft transplantation)

37 χ-square test (2-sided) for intramuscular versus intradermal in the four study groups

38 one-way ANOVA for intramuscular versus intradermal in the four study groups

39 HCs: healthy controls; TNF: tumor necrosis factor; HIV: human immunodeficiency virus; HSCT: hematologic stem cell transplantation; DAS28: disease activity score

Twenty-six HSCT patients were well matched in regard to gender, age, prior influenza vaccination and time since transplantation. Underlying diseases were multiple myeloma (n=10), acute myeloid leukaemia (n=7), non-Hodgkin lymphoma (n=3) and other (n=6). The intradermal group had a higher percentage of patients with an allograft (62 vs 23%, p=0.047).

Immune responses

The intradermal vaccination with a low dose resulted in similar postvaccination titers as compared to standard intramuscular vaccination in all four study groups (HC, anti-TNF, HIV and HSCT group). There was a clear hierarchy in postvaccination titers and protection rates for the different study groups (in both intramuscular and intradermal study arms): healthy controls showed the best responses followed by the anti-TNF group, the HIV group and the HSCT group respectively (Figure 1). Multivariate analyses were done in the four separate study groups.

Prevaccination titers were already protective for a considerable percentage of the subjects (figure 2), which can be caused by either natural exposure or previous vaccination.

The correlation between previous vaccination and higher prevaccination titers was the strongest for the two antigens that were identical to the ones used in the previous (2004/2005) vaccine: influenza A/H1N1 and B (data not shown). Higher prevaccination titers were associated with higher postvaccination titers in all study groups.

In the HIV group, older age and a CD4-count of less than 200 cells per mm³ were associated with lower postvaccination GMTs when controlling for sex, HIV treatment, previous vaccination and prevaccination titers.

In the HSCT group, no prior vaccination and being within the first year post transplantation were associated with lower postvaccination titers (allograft transplantation vs. autograft transplantation only showed a trend for lower postvaccination titers). For healthy

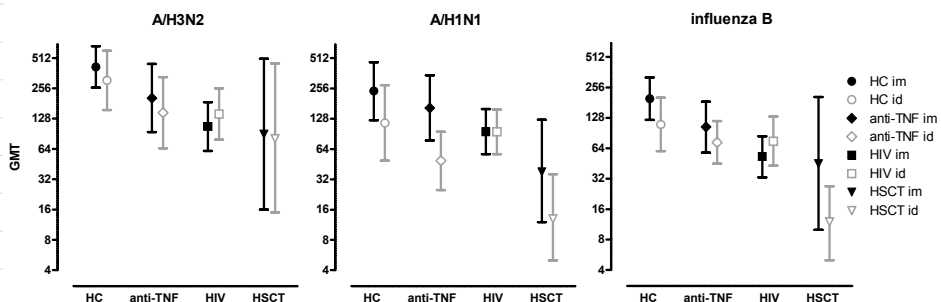


Figure 1. Geometric mean titers at day 28 for respectively influenza A/H3N2, A/H1N1 and influenza B.

Error bars indicate 95% confidence interval.

HC: healthy control; TNF: tumor necrosis factor; HIV: human immunodeficiency virus; HSCT: hematologic stem cell transplantation; im: intramuscular; id: intradermal

* p < 0.05

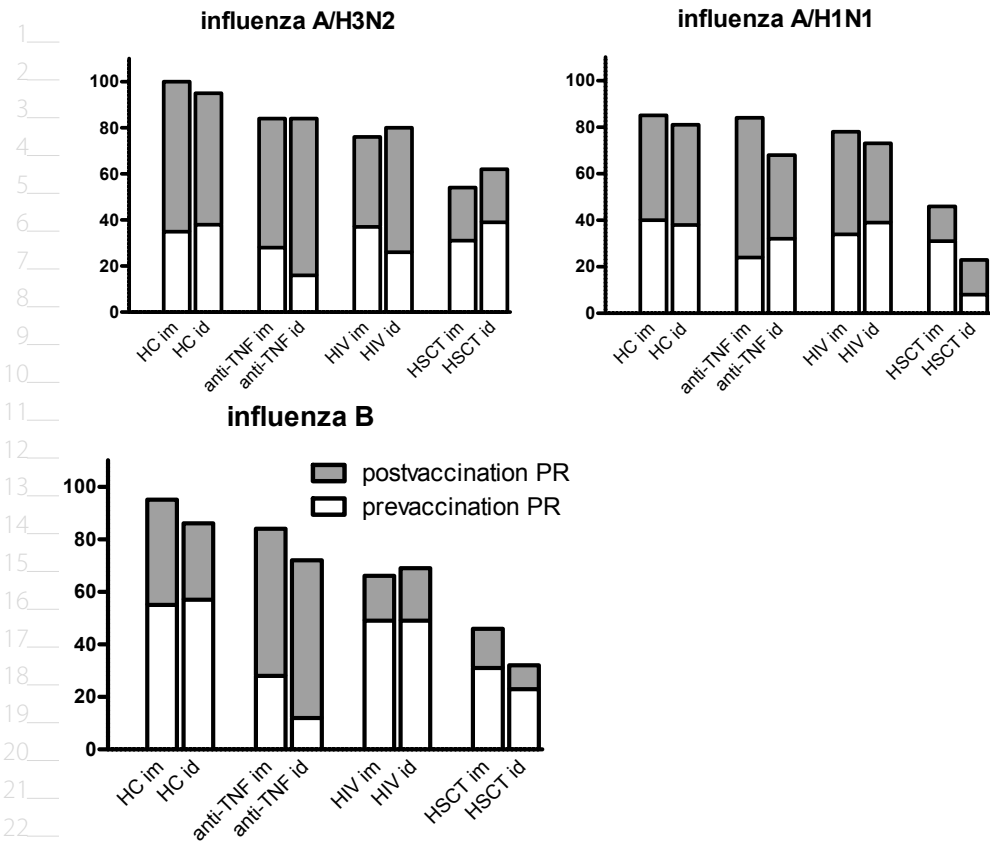


Figure 2. Protection rates before (pre) and 28 days after (post) vaccination for respectively influenza A/H3N2, A/H1N1 and influenza B. P-values from two-sided Pearson's χ^2 -test between groups. HC: healthy controls; TNF: tumor necrosis factor; HIV: human immunodeficiency virus; HSCT: hematologic stem cell transplantation; im: intramuscular; id: intradermal

controls and the anti-TNF group there were no additional factors associated with lower postvaccination titers.

In concordance with the postvaccination titers, the protection rates were similar after intradermal and intramuscular vaccination in the four study groups, for all three antigens. As depicted in Figure 2, the highest percentages of protective titers were seen in healthy controls (81-100%, depending on vaccination route and antigen). Protection rates were lower in anti-TNF and HIV patients (66-84%) and lowest in HSCT patients (23-62%).

All of the healthy controls responded to at least one antigen and 76% to all three antigens contained in the vaccine (Figure 3). In contrast, 42% of all HSCT patients did not respond to any antigen and only 27% responded to all three. Data for the other study groups are depicted in Figure 3.

In a subgroup analysis of patients with the most severe immunodeficiencies, consisting of HIV-infected patients with a CD4 count <200 cells/mm³ (n=10) and HSCT patients

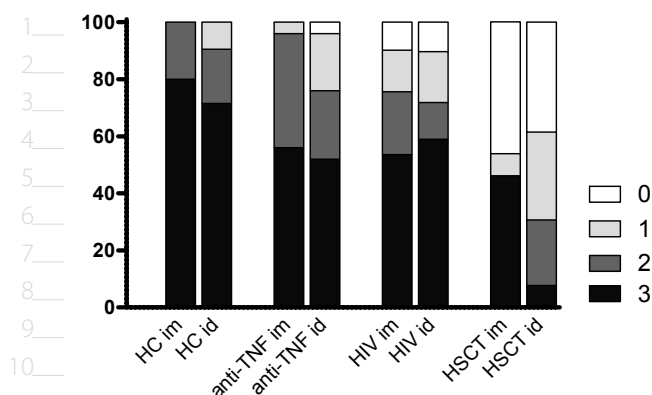


Figure 3. Percentage with a protective titer (≥ 40) against either 0, 1, 2 or 3 of the three vaccine (hemagglutinin) antigens for the different study groups.

HC: healthy controls; TNF: tumor necrosis factor; HIV: human immunodeficiency virus; HSCT: hematologic stem cell transplantation; im: intramuscular; id: intradermal

within the first 11 months following transplantation ($n=11$), the antibody responses were very poor (postvaccination GMTs of 21, 26 and 13 for respectively A/H3N2, A/H1N1 and influenza B). Of the 21 patients, 6 were vaccinated before and received intradermal vaccination. These six patients yielded remarkable protection rates for patients with a severe immunodeficiency (67-83% (depending on the antigen) vs. 13-27% in the 15 patients not vaccinated before and/or vaccinated intramuscular, $p < 0.05$ for A/H3N2 and influenza B; $p=0.09$ for A/H1N1).

Safety and adverse reactions

The mean diameter of the wheal caused by the intradermal vaccination was 7.6 mm (SD 1.6 mm), with no significant differences between the study groups. Skin atrophy caused by corticosteroid use in the past and a very dark skin colour made the technique of intradermal vaccine delivery more difficult.

To exclude a recall bias, only study logs that were returned within three months after vaccination were included in the safety analysis. Sixty three percent of the participants ($n=125$) returned the study log and recorded whether or not they had suffered adverse reactions.

No serious adverse reactions were experienced in the present study. The frequency of adverse reactions after intramuscular vaccination (ranging from muscle pain to fever) reported in this study was high (11-48%). Local reactions, mostly consisting of a transient painless erythema, 48 hours after intradermal vaccination, occurred frequently in the intradermal groups, however significant more often in healthy controls than in immunocompromised patients ($p < 0.05$ Pearsons chi square test, Table 2). The frequency of reported local reactions after intradermal vaccination reflected antibody responses after

Table 2. Percentage of subjects that reported side effects following vaccination within each study group. P-values from comparison against healthy controls (Pearson chi-square test).

study group	im	p-value	id	p-value
HCS	18	reference	89	reference
anti-TNF	48	ns	58	ns
HIV	19	ns	55	ns
HSCT	11	ns	25	.01
ICHs (total of three patient groups)	30	ns	52	.038

HCS: healthy controls; TNF: tumor necrosis factor; HIV: human immunodeficiency virus; HSCT: hematologic stem cell transplantation

ICH: immunocompromised hosts

vaccination and was the highest in healthy controls, followed by the anti-TNF group, HIV group and HSCT group respectively (Table 2).

Of the subjects who did not respond to any of the antigens after intradermal vaccination, only one subject reported a local skin reaction. In contrast, all healthy controls (and 69% of immunocompromised patients) who responded to all three antigens reported a local skin reaction. Local skin reactions were predictive of a response to at least one out of three antigens (paired samples t-test, $p < 0.05$), with a sensitivity of 63% and a specificity of 83% for all subjects pooled.

DISCUSSION

Intradermal influenza vaccination, using only a fifth of the normal dose, leads to similar postvaccination antibody titers and protection rates as compared to standard intramuscular vaccination in immunocompromised patients. Since there is a dose-response relation between the amount of antigen used and the subsequent serologic response, this indicates that intradermal vaccination is more efficient in inducing antibody responses than intramuscular vaccination. [17, 18] This principle was recently proven to be valid in a clinical study. [36]

A likely mechanism for this phenomena is that the abundance of antigen presenting cells (APCs) in the dermis reduces the chance that antigens are cleared by circulating pre-existing antibodies or non-specific immunity after intradermal vaccination. In animal studies, intradermal vaccination induced a faster and more profound cellular immune responses in the local lymph node than intramuscular vaccination. [37] Earlier studies that compared reduced dose intradermal influenza vaccination with standard intramuscular vaccination have been criticized for not including a third study arm with a low dose (0.1 mL) delivered intramuscular. [38] Since we anticipated significantly lower protection rates in patients indicated to receive influenza vaccination, such a study arm was not considered to be ethical. [16]

Patients who, as a result of cellular immunodeficiency, respond with lower postvaccination titers and protection rates to T-cell-dependent vaccines such as the influenza vaccine have the most to gain from a more efficient immune response. [5-7] The underlying T-cell and APC defects, different in nature and severity in the three patient groups of the present study, can be partially overcome by using intradermal vaccine delivery. [39-41] In the present study, only the HIV-group had higher titers after intradermal vaccination. Whether or not this reflects a true different (more efficient) mechanism as opposed to the other study groups is not clear from the present study. The only other study that reported on intradermal influenza vaccination in HIV-infected patients also found a trend for a higher percentage of responders in subjects receiving intradermal vaccination than in those receiving standard intramuscular vaccination. [29]

Several vaccines have been proven to be safe and effective when a reduced dose was delivered intradermally, mostly in immunocompetent subjects. [42-46] Intradermal vaccination in immunocompromised patients has been studied extensively, mostly using the hepatitis B virus (HBV) vaccine. A meta-analysis of intradermal HBV vaccination in patients with chronic kidney disease showed higher protection rates after intradermal administration of the vaccine than after standard intramuscular vaccinations. This difference was no longer detected during the follow up (6-60 months after completion of the vaccination schedule). [42]

Although in theory intra-dermal delivery of vaccines could save up to 240 million of the 300 million influenza vaccine doses distributed annually worldwide, this is not likely to be a realistic scenario. [31] The more demanding administration technique will hamper mass vaccination campaigns and the frequency and severity of adverse effects following intradermal vaccination in immunocompetent subjects are such that this technique is unsuitable for routine influenza vaccination. Immunocompromised patients, however, form a relatively small subgroup of those indicated for annual influenza vaccination and adverse reactions are significantly milder and less frequent in these patients than in healthy subjects. The overall frequency of adverse reactions might be overestimated in the present study, since a proportion of the subjects who did not return their study log, reported that they did so because they had no adverse events to report.

An interesting finding in the present study was that the presence of a local skin reaction correlated with the magnitude of the antibody response to at least one out of the three vaccine antigens. The absence of a local skin reaction within the first 48 hours following vaccination identified patients that did not develop an adequate response (measured 4 weeks later). In our study this subgroup was too small to evaluate the effect of booster vaccination. The skin reaction was interpreted as a delayed type hypersensitivity reaction, which could be directed at either of the three hemagglutinin or neuraminidase antigens or even the traces of chicken egg or preservatives contained in the vaccine.

1__ Of further interest were the remarkably high postvaccination protection rates after
2__ intradermal vaccination in a subset of severely immunocompromised patients who were
3__ vaccinated before. A greater effect of intradermal vaccination in a primed population
4__ as compared to a 'virgin' population was already reported 50 years ago. [20] Again the
5__ subset of (combined HIV and HSCT) patients was too small in the present study to draw
6__ any firm conclusions. Still, the fact that up to 83% of the primed patients who received
7__ intradermal vaccination, had protective postvaccination titers suggests that intradermal
8__ vaccination could be the most optimal route of (annual) vaccination in patients with a
9__ severely impaired cellular immunity.

10__ In conclusion, dose sparing intradermal influenza vaccination is a feasible alternative
11__ for the routine practice of intramuscular vaccination in several groups of immunocom-
12__ promised subjects, including patients treated with anti-TNF, HIV-infected patients and
13__ HSCT patients. In these patients the local skin reaction upon vaccination may be used
14__ as a predictor for the outcome of vaccination, identifying the patients who most likely
15__ would benefit from a booster vaccination. Intradermal vaccine delivery should be further
16__ explored, especially for annual influenza vaccination of severely immunocompromised
17__ patients.

18__

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24__ valuable contribution to the statistical analysis.

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Appendix | 2

Appendix to chapter 3 and 4 RICH-2 study - additional data

Unpublished supplemental data

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1 Background

2 The original study protocol included a cross-over design with an influenza re-vaccination
3 at week 4. The reason for this re-vaccination was to minimize the risk of underprotection
4 in immunocompromised patients, who received a reduced dose vaccination. The reason
5 to re-vaccinate already at week 4 was mostly practical: in the RICH-1 study the number
6 of patients lost to follow up increased with every time point, further more we aimed to
7 achieve maximal protection in time for the influenza season.

9 Wheal size, side effects and immune response

10 As a quality control wheal size was measured after every intradermal vaccination (Figure
11 1a). A wheal size of greater than or equal to 6 mm was considered adequate. This was
12 achieved in all but 3 subjects (Figure 1b). The final analysis was done on a intention-to-
13 treat base, however excluding these three subjects from the analysis did not alter outcomes.
14 Unfortunately, we did not systematically ask subjects to measure the size of the postvac-
15 cination skin rash. Especially in healthy controls, a local reaction of more than 5 cm
16 in diameter was frequently noticed (Figure 1c). As noted in the article, frequency and

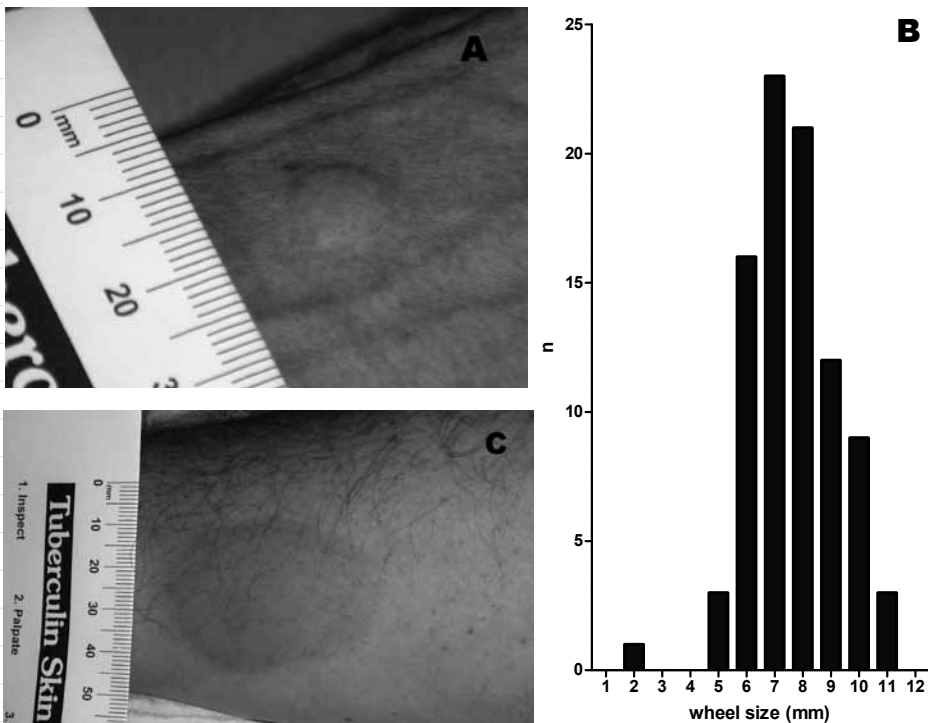


Figure 1a. Typical wheal (diameter 8 mm) after intradermal vaccination in a HIV-infected patient with lipo-atrophy. **1b.** Histogram of wheal size in all subjects. **1c.** Painless local skin reaction (maximum diameter 55 mm) in a healthy control 48 hours after intradermal vaccination.

severity of the local skin reaction was related both to the severity of the immunodeficiency and the subsequent antibody response.

Longterm follow up

At four weeks subjects randomized to the intradermal vaccination (id) arm received a regular intramuscular (im) vaccination and subjects randomized to the im arm received a reduced dose id vaccination. Antibodies were measured at week 0, 4, 8 and 26 (for a subgroup) as presented in Figure 2. In the original publication only the week 4 data are represented graphically for reasons of clarity.

The HIV-groups were the only groups that met the predefined size from the power calculations (90% power to detect a 50% difference in post vaccination GMT in the id versus the im group); the other study groups are used as reference groups.

Revaccination at week 4 did not give a true booster response. Titers did increase in all study groups (except for the rituximab treated patients), but the effect on the protection rate was modest or even absent.

Anti-influenza titers remained stable six months after repeated vaccination in healthy controls but not in HIV-infected individuals. This information is relevant in case of 'late' influenza epidemics (as occurred in The Netherlands 2006 and 2007) and in case of cross-equator travel, in which cases HIV-infected individuals might be unprotected. Protection rates, 6 months after repeated vaccination, were as low as 67, 48 and 29% for influenza A/H3N2, A/H1N1 and B, respectively (vs. 100, 71 and 86% in healthy controls; pooled data from im and id vaccination). Protection might even be more short lived in subjects who received only a single vaccine dose.

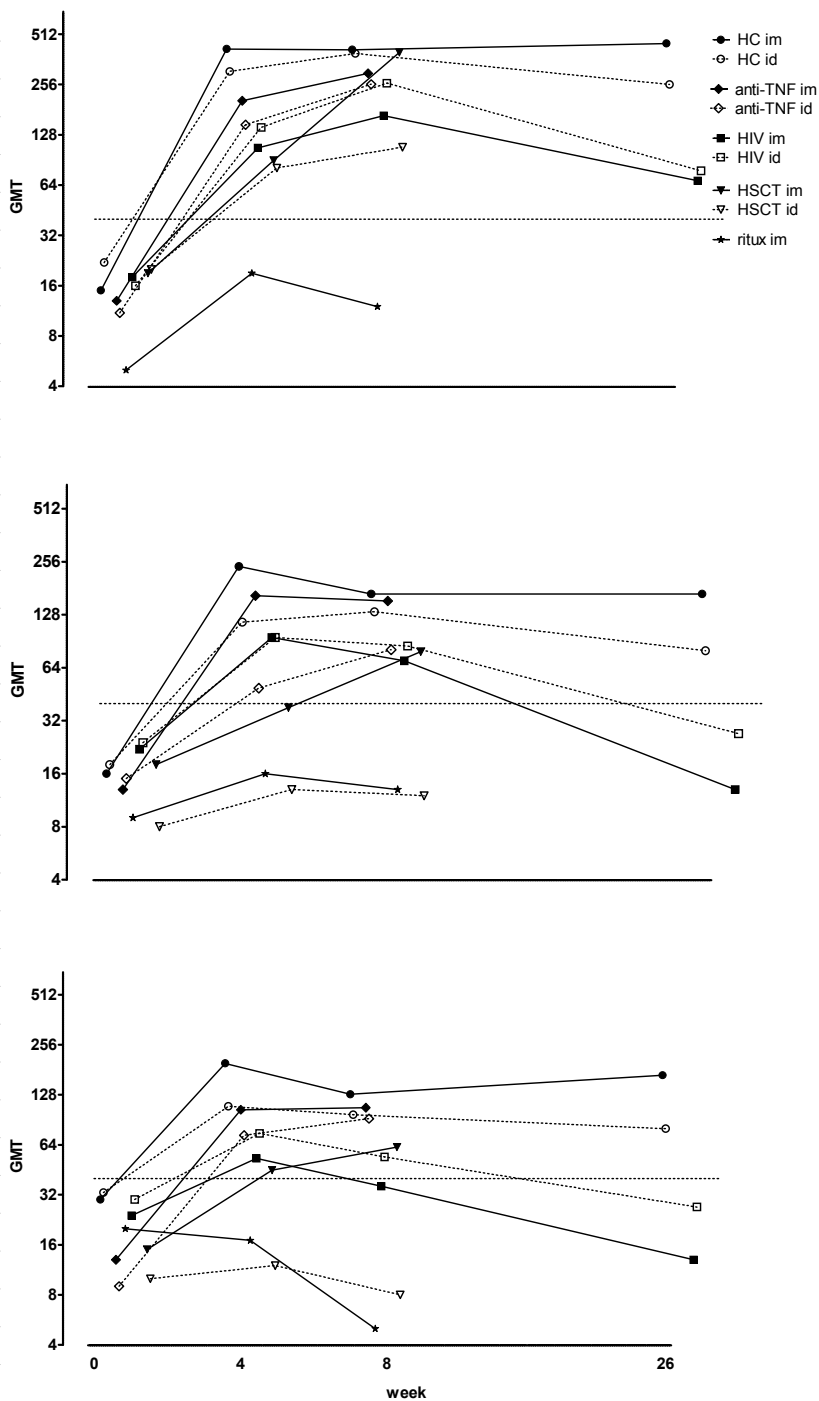


Figure 2. Geometric mean titers (95% confidence interval) against influenza A/H3N2, A/H1N1 and influenza B at the different time points.

Chapter | 5

The induction of cross-reactive antibodies after influenza vaccination is not impaired in HIV-infected individuals

submitted

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1 ABSTRACT

2
3 **Background.** The composition of influenza vaccines is updated annually to match circu-
4 lating epidemic strains. However, influenza vaccination induces antibodies that cross-react
5 with antigenic drift variants to a certain extent. We analyzed the cross-reactivity of serum
6 antibody responses after influenza vaccination of human immunodeficiency virus (HIV)-
7 infected patients and healthy controls.

8 **Methods.** Sera of 38 HIV-infected patients and 18 healthy controls who were vaccinated
9 with the 2005/2006 trivalent influenza vaccine were tested for the presence of antibodies
10 against the homologous influenza A/H3N2 vaccine strain and vaccine strains used in
11 subsequent years (2005-2009). Serum antibody titers were determined using the hemag-
12 glutination inhibition (HI) assay. Geometric mean titers (GMTs) are reported as primary
13 outcome.

14 **Results.** Antibodies elicited by influenza vaccination cross-react with drift variants. This
15 phenomenon is, as expected, dependent on their antigenic distance. The level of cross-
16 reactivity of anti-influenza antibodies is comparable in HIV-infected individuals and
17 healthy controls.

18 **Conclusions.** HIV-infected individuals, like healthy controls, are likely to benefit from
19 the cross-reactivity of influenza virus specific antibodies elicited by influenza vaccination
20 when the vaccine strain does not completely match the epidemic strain.

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1 BACKGROUND

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3 Influenza viruses are a major cause of annual outbreaks of respiratory disease. [1-2] These
4 viruses display considerable antigenic drift in particular in the hemagglutinin, which is
5 driven by antibodies induced by previous infections or vaccination in the general popula-
6 tion. [3-5] Consequently, new variants emerge that escape recognition by virus neutral-
7 izing antibodies. The emergence of antigenically distinct influenza A/H3N2, A/H1N1
8 and B viruses necessitates the update of the vaccine composition almost annually. [6-9]
9 Annual influenza vaccination is recommended for persons at risk for complications of
10 infection, including immunocompromised patients. [10]

11 The extent of cross-reactivity of vaccination-induced antibodies with antigenic drift
12 variants depends on several factors most notably the antigenic distance between the vac-
13 cine strains and the respective epidemic variant strains. [11-13] Specific anti-influenza
14 antibody producing memory B-cells can be found in the blood, decades after exposure to
15 a specific strain. [14] Protection against distinct influenza strains can occur after natural
16 influenza infection; the induction of cross-reactive antibodies may also afford some level
17 of protection against deviant influenza virus strains. [15-18] For example, the induction of
18 antibodies against the neuraminidase of an influenza A/H1N1 virus even afforded protec-
19 tion against infection with an A/H5N1 influenza virus in mice. [19]

20 The high attack rate of the 2009 influenza A/H1N1 virus is facilitated by the scarce pres-
21 ence of (cross-reactive) antibodies against this strain in the general population. [20-21]
22 Cross-reactive antibodies against this new influenza virus have been found in a proportion
23 of elderly subjects, most notably those born in the first decades of the twentieth century,
24 probably as a result of infection with the antigenically similar 1918 pandemic influenza
25 A/H1N1 strain, more than 9 decades before, or the descendant viruses of this strain.
26 [22-24] These antibodies provide some clinical protection against the 2009 influenza A/
27 H1N1. [25] Strategies to profit from the cross-reactivity of anti-influenza antibodies are
28 being developed. [26-30]

29 It has been shown that in the elderly (≥ 75 years of age) the antibody response induced
30 by vaccination was less cross-reactive than that of younger subjects, which is thought to be
31 caused by immune senescence. [31] This could make this age group more susceptible to
32 infection with epidemic influenza virus strains that do not completely match the vaccine
33 strains. If this reduced cross-reactivity is indeed caused by immune senescence, we would
34 expect to find this phenomenon in HIV-infected individuals as well. Chronic HIV-1
35 infection causes immune activation and a generalized inflammatory state, characterized by
36 high numbers of activated CD8⁺ lymphocytes. This T-cell activation causes an accelerated
37 immune senescence in HIV-1. [32-34]

38 To assess the cross-reactivity of vaccine-induced antibody responses of HIV-1 infected
39 subjects, the reactivity of post vaccination sera of HIV-1 infected individuals and healthy

controls subjects vaccinated with a 2005/2006 influenza vaccine was tested with influenza viruses that circulated in subsequent years.

METHODS

Trial design and subjects

The HIV-1 infected patients (HIV group) and control group (HC group) included in the current study are a selected subset of subjects from a previous study. [35] Subjects with a prevaccination titer <40 and a post vaccination titer ≥ 40 to influenza A/H3N2 were included in this sub study. To exclude an influence of pre-existing anti-influenza antibodies, subjects with higher prevaccination titers were excluded. The exclusion of non-responders (a postvaccination titer <40) follows from the fact that the cross-reactivity of anti-influenza antibodies could only be determined in subjects with a measurable antibody titer. The study protocol was approved by the appropriate institutional ethics committees (LUMC, local CME number P05.115; ISRCTN15762138) and conducted in accordance with the Declaration of Helsinki. All participants provided a written informed consent.

Vaccine and vaccination

All study subjects were vaccinated in the fall and winter of 2005 with a commercially available trivalent subunit influenza vaccine (Influvac™ 2005/2006, Solvay Pharmaceuticals B.V., Weesp, The Netherlands) at day 0. The vaccine contained 15 μg of hemagglutinin of each of the following strains: A/California/7/04 (H3N2)-like strain (vaccine strain NYMC X-157) further referred to as A/H3N2/California or homologous X-157 strain; A/New Caledonia/20/99 (H1N1)-like strain (vaccine strain IVR-116) and B/Shanghai/361/02-like strain (B/Jiangsu/10/03).

Vaccines were stored at 6°C and administered at room temperature and given either intramuscular (0.5 mL) according to the package insert or intradermal (0.1 mL) as described before. [35]

Antibody assays and statistics

Serum samples were collected at day 28 and were stored at -80°C until use. The hemagglutination inhibition (HI) test was performed in duplicate according to standard methods with turkey erythrocytes and four hemagglutinating units of virus to measure antibodies against each of hemagglutinins as described before. [35] We tested antibodies against influenza A/H3N2 viruses since these subtypes displayed the highest degree of antigenic drift. The A/H3N2 strains used in the HI assay were X-157 (the A/California/7/04-like vaccine strain for the 2005/06 season: the homologous vaccine strain), and the heterologous vaccine strains X-161b (the A/Wisconsin/67/05-like vaccine strain for the 2006/07 and

2007/08 seasons) and X-175c (the A/Brisbane/10/07-like vaccine strain for the 2008/09 and 2009/10 seasons). The antigenic distance between X-157 and X-175c is larger than the antigenic distance between X-157 and X-161b. Ferret sera raised against the test antigens were used as positive controls against all antigens used in this analysis. All sera were tested simultaneously, which included retesting all sera against the homologous vaccine strain. For statistical analysis a titer of 5 was arbitrarily assigned to sera with a titer <10. Titers were transformed to a logarithmic scale and geometric mean titers (GMTs) were used for further calculations. Crude GMTs are reported for all antigens tested.

The results were analyzed by multivariate logistic regression (ANOVA) analysis, using natural logarithms of HI titers. A p-value <0.05 was considered to indicate a statistical significant difference between groups. Calculations were performed using SPSS for Windows, version 15.0.

RESULTS

Baseline characteristics

Baseline characteristics of the study subjects are summarized in Table 1. The route of vaccination, either intramuscular (im) or intradermal (id), did not influence post-vaccination titers in the original, or in the present study. [35] Therefore, data obtained from im and id vaccinated subjects were pooled.

Table 1. Baseline characteristics of subjects.

characteristics	HC	HIV	p-value
n	18	38	
male,%	56	71	ns †
age, mean, years (range)	44 (20-69)	45 (22-75)	ns ‡
previously vaccinated,%	0	61	<0.0001 †
vaccination route:% intradermal	44	53	ns †
CD4, median, cells/ μ L (inter quartile range)	-	444 (348-638)	-
HAART,%	-	71	-

† χ^2 -square test (2-sided); ‡ one-way ANOVA

HC: healthy control group; HIV: human immunodeficiency virus group

Antibody titers

HI titers against the homologous vaccine strain, X-157, were tested in 2006 (for the original study) and again for the present study. There was a good reproducibility of the original data with a correlation coefficient of 0.90 ($p < 0.001$, Spearman's rho).

Figure 1 shows GMTs against the homologous and heterologous strains in the two study groups. As reported earlier, the (quantitative) antibody response upon influenza vaccination is impaired in HIV-infected individuals compared with healthy controls. [35] With an increasing antigenic distance, decreased cross-reactive antibody titers were found in both groups.

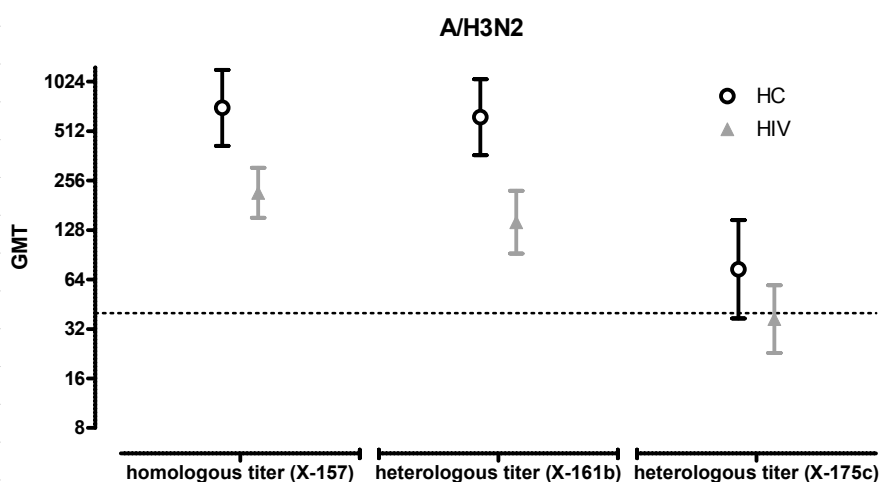


Figure 1. Geometric mean titers (GMT) against homologous and heterologous (influenza A/H3N2) vaccine antigens for the two study groups.

Whiskers indicate 95% confidence interval; dashed line indicates protection threshold (40).

HC: healthy control group; HIV: human immunodeficiency virus group

Figure 2 shows a scatter plot of the (natural logarithm of the) homologous titer X-157 on the x-axis versus the (natural logarithm of the) heterologous titers X-161b (panel A) and the more distant X-175c (panel B) on the Y-axis. The regression lines plotted in panel A do not differ significantly between the two study groups. In panel B there is a statistically significant difference between the two regression lines, indicating that the absolute decrease in cross-reactive HI titers was stronger in the healthy control group as compared with the HIV group (ANOVA $p < 0.005$). The proportional decrease of the antibody titers to X-175c compared to the homologous titer to the X-157 strain was 35% (SD=16) and 34% (SD=20) in healthy controls and HIV-infected individuals respectively, which is not a statistically or clinically significant difference.

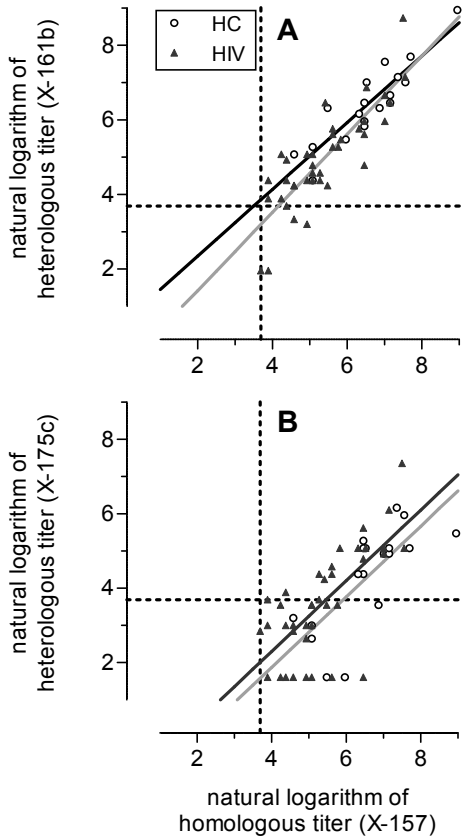


Figure 2. Scatterplot of (natural logarithm of) homologous titers against (natural logarithm of) the two heterologous titers (panel A and B) for the two study groups, including regression lines. Black line: HC; grey line: HIV
HC: healthy control group; HIV: human immunodeficiency virus group
Difference between the two regression lines significant in panel B (ANOVA, $p < 0.005$).

In a multivariate regression analysis we did not find an effect of age, gender, vaccination route, (nadir) CD4 T-cell count or previous vaccination on the forming of cross-reactive antibodies.

In the present study, only previously unvaccinated healthy controls were included, because most healthy controls who were vaccinated the year before fulfilled the exclusion criterion of a prevaccination HI titer ≥ 40 . Within the HIV-group, titers a year after (previous) vaccination were lower allowing for inclusion of both previously unvaccinated and vaccinated patients. Within the subset (61%) of previously vaccinated HIV-infected individuals, we found slightly higher pre- and postvaccination titers. The exclusion of previously vaccinated individuals did not significantly influence our findings.

DISCUSSION

In the present study we show that the cross-reactive properties of the virus specific antibody response of HIV-infected patients, with a median CD4 count of 444 cells / micro-liter, upon influenza vaccination, are comparable to that of healthy control subjects. This preserved qualitative response contrasts with the impaired quantitative antibody response in these patients compared with healthy controls, as was reported in an earlier study. [35] The present study was done with a subgroup of the study participants (responders) from that study.

Although the composition of the influenza vaccine is updated yearly, based on antigenic and epidemiological data of circulating epidemic strains, sometimes the vaccine strains do not match the epidemic strains completely. [36] Still, antibodies elicited by vaccination do cross-react with these antigenic drift variants and may afford some degree of protection against infection and are therefore a clinically relevant phenomenon. The reason to evaluate the cross-reactive properties of postvaccination antibodies in HIV-infected individuals were findings in healthy subjects older than 75 years, reported earlier. [31] In the elderly the quantitative antibody response upon influenza vaccination is also impaired. [37] Moreover, the induction of antibodies that can cross-react with newly emerging antigenic variants was reduced in subjects >75 years of age. [31] This would render this age group more at risk of morbidity and mortality due to influenza, when novel antigenic variants emerge that do not match the vaccine strain. The impaired quantitative antibody response of older individuals is most likely caused by immune senescence resulting in impaired virus specific T- and B-cell responses. [38-39] Accelerated immune senescence is also one of the mechanisms behind the impaired immune response found in HIV-infected individuals. [32-34] At present it is unclear why especially the cross-reactive antibody response (a qualitative aspect of the immune response) is affected in the elderly (>75 years of age) but not in HIV-infected individuals. It might well be that the immunological damage in persons aged over 75 years is far greater than in our study population of HIV-infected individuals. High age is associated with low numbers of naïve B- and T-cells, most notably resulting in poor responses upon neo-antigens. Concurrently there are high numbers of activated T- and B-cells, creating a pro-inflammatory environment in high age. The combination of scarce naïve cells and pre-existing B- and T-cell clones to older influenza antigens, might skew the antibody response towards older antigenically related strains, a phenomenon known as original antigenic sin. [40] It was demonstrated recently that antibodies directed to historic A/H1N1 viruses, cross reacted with the 2009 influenza A/H1N1. These antibodies were detected in a substantial proportion of subjects born in the first decades of the twentieth century. [23-24]

1 In the present study, the cross-reactivity of the antibodies formed upon vaccination was
 2 not correlated with the (nadir) CD4 cell count, gender or age. Of note, most patients
 3 were effectively treated with antiretroviral treatment and severely immunocompromised
 4 patients, with a CD4 count of less than 200 cells / microliter, were almost absent in
 5 the present study. We cannot exclude that severely immunocompromised HIV-infected
 6 individuals do show decreased cross-reactivity.

7 None of the study participants was older than 75 years. Other studies did not report
 8 an effect of older age on cross-reactivity either. However these studies, like our own study,
 9 included less old subjects in their analysis. [41-42]

10 Sixty one percent of HIV-infected individuals was also vaccinated in the year before the
 11 study vaccine was administered (2005/2006), as recommended by guidelines, that advice
 12 annual influenza vaccination. In 2004/2005 the influenza A/Fujian/411/02-like strain
 13 was used, which is different from the vaccine strain tested in the present study. The fact
 14 that we reported higher prevaccination titers in subjects who were previously vaccinated
 15 already signifies the cross-reactive potential of antibodies formed upon influenza vaccina-
 16 tion. [35] Because we aimed to study the cross-reactivity of newly formed antibodies
 17 upon vaccination, we included only subjects with a prevaccination titer <40 in the present
 18 study. Within this selected population for the present study, we found no effect of previous
 19 vaccination on study outcomes.

20
 21 In conclusion, virus specific antibodies induced after influenza vaccination of HIV-infected
 22 individuals, effectively treated with antiviral drugs, do not have impaired cross-reactive
 23 properties compared with those induced in immunocompetent individuals. This may
 24 be of clinical relevance for HIV-infected individuals, when the epidemic strain does not
 25 completely match the vaccine strain.

26

27 *Acknowledgments*

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 29 thank Willemien Dorama, HIV care and research nurse, LUMC, for her contribution
 30 to the collection of materials; Ruud van Beek, research analyst, Erasmus Medical Center,
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 32 Medical Center, Rotterdam, for his advice on the statistical analysis.

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Chapter | 6

Restoration of T-cell mediated immune response upon rabies vaccination in HIV-infected patients treated with HAART

AIDS. 2009;23:2451-8.

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1 ABSTRACT

2
3 **Design.** Rabies vaccine was used as a T-cell-dependent neo-antigen to investigate several
4 aspects of the primary and booster immune response *in vivo* in HIV-infected individuals
5 receiving antiretroviral treatment.

6 **Methods.** Subjects received rabies vaccination twice, with a three months interval. Serum
7 samples were taken before and 1, 2 and 4 weeks after both vaccinations and 1 and 5 years
8 after the primary vaccination. Anti-rabies antibodies (IgG, IgG subclasses, IgA and IgM)
9 were determined; antibody avidity was measured after both vaccinations. T-cell subsets
10 were characterized by flow cytometry.

11 **Results.** Eighteen healthy controls and thirty HIV-infected adults, treated with HAART
12 for almost 4 years, with a median CD4+ T-cell count of 537 cells per μL were immunized.
13 The post vaccination concentrations of anti-rabies IgG and IgM were significantly lower
14 in HIV-infected individuals as compared to controls. Three T-cell-dependent processes,
15 a true booster response, a class switch from IgM to IgG and avidity maturation, were
16 present in both healthy controls and HIV-infected individuals. Higher age was associated
17 with lower post-vaccination anti-rabies IgG and IgM titers. Five years after the primary
18 vaccination, 63 percent of the HIV-infected individuals still had antibody titers above the
19 protection threshold.

20 **Conclusion.** Immune restoration in HIV-infected individuals treated with HAART, re-
21 sulting in a CD4+ T-cell count greater than 500 cells per μL , is incomplete. However, the
22 majority of HIV-infected individuals is capable to mount a long lasting immune response,
23 including several pivotal T-cell-dependent processes, upon vaccination with a neo-antigen
24 like the rabies vaccine.

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1 INTRODUCTION

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3 Highly active anti-retroviral therapy (HAART) effectively suppresses HIV replication,
4 resulting in diminished immune activation and functional immune recovery of both T-
5 and B-lymphocytes, reflected in a decline in morbidity and mortality due to opportunistic
6 diseases. [1-6] The increase in number of peripheral blood CD4+ T-cells after HAART
7 initiation is a composite of an initial redistribution of predominantly memory cells from
8 lymph nodes followed by a continuous slow repopulation with naïve T-cells. [7,8] Within
9 several months after initiation of HAART partial recovery of the CD4+ T-cell repertoire
10 is observed. It has not yet been well established to what extent the quantitative increase
11 in CD4+ T-cells is a reflection of reconstitution of the qualitative immune function in
12 HIV-infected individuals. [9] Several studies conducted in the HAART era have shown a
13 functional but incomplete recovery of T-cell mediated immunity after HAART initiation
14 in HIV-infected individuals. [10-13]

15 Vaccination with a T-cell-dependent neo-antigen like the rabies vaccine is an elegant
16 approach to study various aspects of the humoral and cellular immunity *in vivo*, since
17 antibody production, and features of memory responses such as class switch from IgM
18 to IgG antibodies, the forming of a booster reaction (a logarithmic titer increase within
19 days after re-exposure to the antigen) and avidity maturation (clonal selection of the most
20 specific antibodies) all are T-cell-dependent processes. [14]

21 We studied these responses in chronically HIV-infected individuals, who were on treat-
22 ment with HAART for at least eight months and compared the response to that of healthy
23 controls.

26 METHODS

28 Study cohort

29 HIV-1-infected adults from the Infectious Diseases outpatient clinic of the Leiden
30 University Medical Center, treated with HAART for at least 6 months, were invited to
31 participate in this study. Healthy adults, with no risk factors for HIV, served as controls.
32 All participants were naïve to the rabies vaccine. The protocol was approved by the lo-
33 cal ethics committee (local protocol number P98.220). A written informed consent was
34 obtained from all participants.

36 Vaccine

37 All subjects received 1.0 mL of a commercially available rabies vaccine (human diploid
38 cell vaccine (HDCV), containing inactivated rabies virus (Wistar PM/WI 38-1503-3M
39 strain), Pasteur Mérieux MSD, Lyon, France) with a potency of 2.5 IU/mL, at day 0

(primary vaccination) and week 12 (booster vaccination), administered intramuscular in the deltoid region, according to the package insert. [15] The rabies vaccine was chosen because it represents a true neo-antigen and the immune response upon this antigen is T-cell-dependent. The vaccination schedule used in the present study includes fewer doses than routine pre- or post-exposure schedules (consisting of either 3 doses at days 0, 7 and 21 (28) or 5 doses at days 0, 3, 7, 14 and 28, using 1.0 mL on each occasion). The 0 and 3 months schedule was preferred in the present study because it represents a more optimal timing for priming (a process that takes far more time than 3 or 7 days) and subsequently a true booster response.

ELISA for quantification of antibodies against Rabies

Blood samples for serological follow up were taken at 0, 1, 2, 4 weeks after primary and booster vaccination, which were 12 weeks apart, and 1 and 5 years after the primary vaccination. Sera were stored at -20°C until use; all serum samples from the first 16 weeks were tested at once, the sera from the one and five year follow up were tested at a later point in time.

Anti-rabies antibodies were quantified by a rabies specific enzyme linked immunoassay (ELISA) as described before, using labeled polyclonal antibodies for detection of IgG, IgA and IgM and monoclonal antibodies for detection of IgG1 and IgG3. [16]

Although this ELISA does not measure neutralizing antibodies per se, there is a high correlation between the antibodies measured by ELISA and neutralizing antibodies and WHO, CDC and local agencies endorse the use of ELISA in stead of the more elaborate neutralizing antibodies measurements for the assessment of vaccine efficacy. [17,18] Furthermore, our primary end-goal was an immunological comparison between groups, for which both tests are equally suitable.

Avidity testing

The avidity of anti-rabies IgG subclasses was measured by a modified elution ELISA as described before. [16] The relative avidity index (AI) was defined as the molarity of NaSCN at which 50% of the amount of anti-rabies IgG subclasses remained bound to the coated rabies antigen.

T-cell subsets

Phenotypic analysis of T-cell subsets was done by flow cytometry (FACSCalibur, Becton, Dickinson Biosciences, San Jose, CA, USA). CD4⁺ and CD8⁺ lymphocytes were characterized as either naïve cells (CD45RA⁺), memory cells (CD45RO⁺) or double positive cells (CD45RA/RO⁺).

1 Analysis

2 Antibody titers were log transformed for calculations. Seroconversion was defined as a
3 post vaccination IgG anti-rabies antibody titer equal to or greater than 0.5 IU/mL as
4 issued by the World Health Organization (WHO). [19] The protection rate was defined
5 as the percentage within a group with a titer greater than or equal to 0.5 IU/mL.

6 To quantify the booster response, the anti-rabies IgG titer fold increase in the first week
7 following both vaccinations was calculated. A class switch, the relative decrease of IgM
8 antibody in favor of an increase of IgG production after booster vaccination, was arbitrarily
9 defined as a decrease of anti-rabies IgM after booster vaccination as compared to the IgM
10 response after the primary vaccination. Avidity maturation was calculated as the fold in-
11 crease in avidity measured at week 16 versus week 4. A linear regression model was used to
12 identify clinical and immunological parameters correlated with these vaccination outcomes.

13 All analyses were carried out using SPSS software (version 15.0). A two-tailed P value
14 of less than 0.05 was considered to be statistically significant. Separate time points were
15 tested as independent variables.

16

17

18 RESULTS

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20 Study population

21 Thirty HIV-infected individuals and 18 healthy controls were enrolled in this study. Im-
22 munological and clinical parameters of the study subjects are summarized in Table 1;
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24

25 **Table 1.** Basic characteristics of the study population.

	Healthy controls (n=18)	HIV-infected individuals (n=30)
27 Age, mean, years (range)	31 (19-49)	45 (21-71)
28 Gender,% male	50	97
29 CDC stage A / B / C (%)	-	43 / 23 / 33
30 Duration HAART treatment, mean (range), years	-	3.4 (0.7-5.9)
31 CD4+ T-cell count (median (IQR), cells/ μ L)		
- nadir	ND	131 (28-279)
32 - t=0	ND	537 (353-772)
33 CD4+ T-cell phenotype (%)		
- naïve / memory / double	-	32 / 48 / 20
34 HIV-1 viral load, median (range), ¹⁰ log c/mL		
35 - pretreatment	-	5.2 (3.9-6.4)
36 - t=0	-	<1.7 (<1.7-4.5)
37 Percentage with HIV-RNA < 50 c/mL		
- t=0	-	83
38 - t=268	-	87

39 nadir = lowest CD4+ T-cell count in patients history; IQR=interquartile range; ND= not done

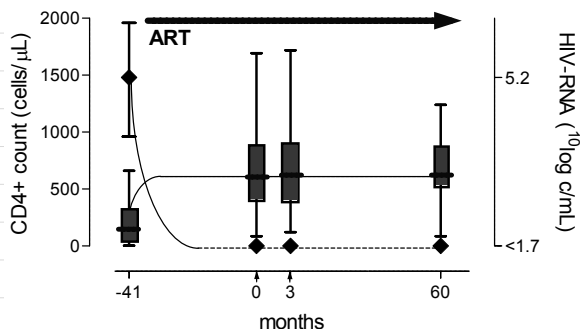


Figure 1. Time schedule of trial, with CD4+ T-cell count (cells/ μ L, line represents median, box represents 25 and 75 percentiles, whiskers represent range) and HIV-RNA (copies/mL, dashed line and diamonds represent median, whiskers (only at $t=-41$ (pre treatment) represent range). Pretreatment values are projected at $t=-41$ months (mean number of months on antiretroviral therapy (ART) at $t=0$). Small arrows on x-axis indicate timing of primary and booster vaccination.

Figure 1 summarizes CD4+ T-cell counts and HIV-1 RNA viral load over time. At the time of primary vaccination the median duration of HAART was 41 months (range 8-71 months). No serious adverse events were reported upon vaccination. One healthy subject showed an allergic reaction (urticariae, clinical diagnosis) ten days after booster vaccination, which subsided without intervention.

All 30 HIV-infected individuals could be included in the 5 year follow up; this last serum sample was drawn after a mean of 268 (range 250-293) weeks, at which time point these patients were on HIV treatment for almost 9 years. HIV-1 RNA concentrations were measured before and one week after both vaccinations and were not influenced by the vaccination. Only a minority of patients had a detectable HIV-RNA load at any time-point during the five years follow-up (respectively 5, 3 and 5 patients at $t=0$, 12 and 268 weeks). Of the five patients with a detectable viral load 5 years after $t=0$, three patients had 'blips' with a transient detectable viral load <150 c/mL and one patient had stopped HIV therapy with a CD4+ T-cell number persisting around 500 cells per microliter. No CD4+ T-cell counts were determined in healthy controls. Eleven of the 18 healthy controls were either lost to follow up or were excluded from the 5 year follow up because they had received a booster vaccination after 1 year as part of a different study protocol. [16]

Antibody titers

The prevaccination concentrations of IgG (Figure 2), both IgG1 and IgG3, but not IgM (data not shown) anti-rabies antibody titers were significantly higher in patients as compared to healthy controls ($P < 0.01$). Post vaccination antibody titers (at all time points) were independent of the prevaccination titer.

The post vaccination concentrations of IgG, IgM and IgA anti-rabies were significantly lower in HIV-infected patients as compared to those in healthy controls ($p < 0.01$, Figure

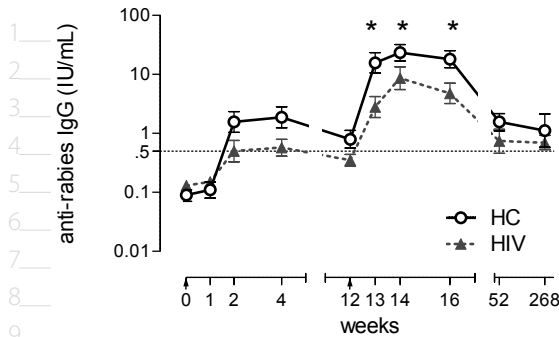


Figure 2. Total anti-rabies IgG titers (IU/mL) in time. Error bars indicate 95% confidence interval and small arrows on x-axis indicate timing of primary and booster vaccination.

* denotes $p < 0.05$ in multivariate regression model, after correction for age.

HC: healthy controls; HIV: HIV-infected individuals.

2; IgM and IgA data not shown), at all time points from week 2 to 4 and week 12 to 16, except for IgM at week 14 and 16, in an univariate analysis.

Both a lower number of naïve CD4⁺ T-cells and higher age were associated with significantly lower (anti-rabies IgG and IgM) titers 2-4 weeks after primary vaccination, but not after booster vaccination in an univariate analysis. Age and the number of naïve CD4⁺ T-cells were inversely correlated (Spearman's rho coefficient -0.55, $p = 0.002$).

In a multivariate regression model, controlling for sex, age and study group (healthy control vs. HIV), only higher age (and not HIV-infection) was associated with lower vaccination titers upon primary vaccination (week 2, 4, and 12). For the booster response (time points 13, 14 and 16 weeks) only HIV-infection was associated with lower anti-rabies IgG titers.

Patients with a detectable viral load at $t = 0$ ($n = 5$) yielded two- to threefold lower anti-rabies (IgG and IgM) concentrations, at all time points from week 2 to 16, as compared to patients with an undetectable viral load. Three out of these five patients only had a low level of viral replication with HIV RNA < 250 copies/mL. The effect of detectable HIV-RNA on anti-rabies IgG titers was independent of the CD4⁺ T-cell count, but statistically significant only at one time point ($t = 14$ weeks) for anti-rabies IgG. The Center for Disease Control classification or the duration of HAART were not correlated with vaccination outcomes.

Individual response patterns varied greatly among the 30 HIV-infected individuals (Figure 3). Analysis of IgG subclass antibodies to rabies showed that the IgG response mainly consisted of IgG1 and IgG3. [16] Seroconversion, defined by the WHO as a post vaccination titer greater than or equal to 0.5 IU/ml, a level considered to be protective against rabies, was achieved in all healthy and HIV-infected subjects after booster vaccination (Figure 4).

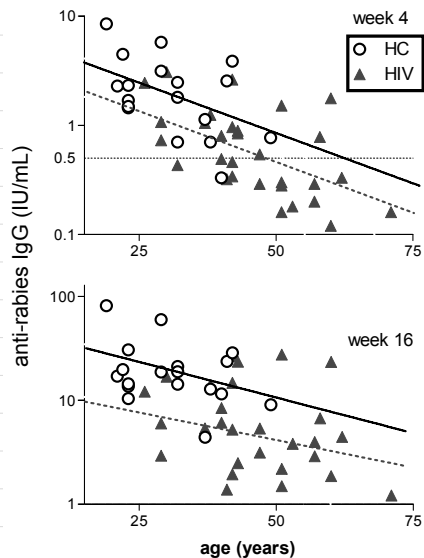


Figure 3. Age versus actual anti-rabies IgG titers (IU/mL) for all individual subjects (open circles: healthy controls; closed triangles: HIV-infected individuals) at week 4 and 16. Pearson's correlation coefficient (r) and p value calculated with log transformed titers. Week 4: HC $r = -0.484$ ($p < 0.05$), HIV $r = -0.573$ ($p < 0.01$); week 16: HC $r = -0.356$ (ns), HIV $r = -0.205$ (ns). HC: healthy controls; HIV: HIV-infected individuals; ns: not significant.

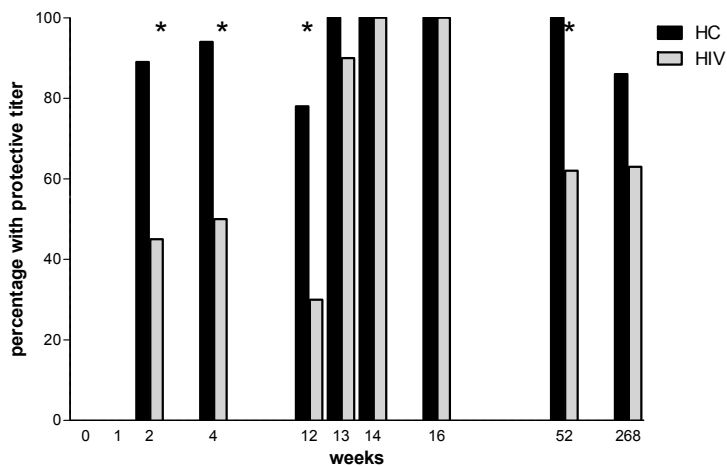


Figure 4. Percentage of subjects with a protective titer (anti-rabies IgG ≥ 0.5 IU/mL) at the different time points (p -values calculated with chi-square; * denotes $p < 0.005$).

Antibody levels tested 5 years after the primary vaccination were still above this protection threshold for six out of seven healthy controls (86%) and for 19/30 (63%) of the HIV-infected individuals tested.

1__ T-cell-dependent processes

2__

3__ *Booster response*

4__ IgG anti-rabies titers increased 24 fold (range 7-64) in healthy controls and 11 fold
5__ (range 1-30) in HIV-infected individuals (ANOVA, $p=0.001$) within the first week after
6__ booster vaccination. Six out of 30 HIV-infected individuals (and nil out of 18 healthy
7__ controls) had a less than 5 fold titer increase. The magnitude of this booster response in
8__ HIV-infected individuals was not related to any of the other clinical or immunological
9__ parameters, such as CD4+ T-cell count, CD4+ T-cell nadir, viral load or age.

10__

11__ *Class switch*

12__ A decrease of the IgM response after revaccination as compared to the IgM response
13__ after primary vaccination was seen in 83% of healthy controls and 60% of HIV-infected
14__ individuals (Pearson's Chi-Square test, $p=0.091$). The probability of such a decreased
15__ IgM response upon booster vaccination in the HIV-infected individuals was significantly
16__ correlated with the total number of CD4+ T-cells at the time of (primary) vaccination
17__ (Spearman's rho $p=0.04$); the median number of CD4+ T-cells in the group that did not
18__ show a class switch was 421 cells per microliter (range 86-803) as compared to 573 (range
19__ 222-1693) in the group that did show a class switch.

20__

21__ *Avidity maturation*

22__ The avidity of IgG1 and IgG3 anti-rabies antibodies in HIV-infected individuals showed
23__ a significant increase at week 16 as compared to week 4, which was stronger in healthy
24__ controls than in HIV-infected individuals. Regarding IgG1 the mean fold increase (stan-
25__ dard deviation) was 1.36 (0.17) for healthy controls and 1.30 (0.12) for HIV-infected
26__ individuals (ns); for IgG3 the mean fold increase (standard deviation) was 1.51 (0.28)
27__ for healthy controls and 1.34 (0.17) for HIV-infected individuals ($p<0.05$ ANOVA). The
28__ increase of avidity was independent of the total number of CD4+ T-cells.

29__

30__

31__ **DISCUSSION**

32__

33__ HIV-infected individuals, who were treated with HAART for more than three years result-
34__ ing in a normalized CD4+ T-cell number, yielded significantly lower post vaccination
35__ titers as compared to healthy controls after booster rabies vaccination. Still, even after
36__ only two vaccinations the protection threshold, as defined by the WHO, was met by all
37__ subjects. Five years after the vaccinations almost two thirds of the HIV-infected individu-
38__ als still had anti-rabies IgG titers above the protection threshold.

39__

Three individual T-cell-dependent processes, which add up to an overall functional quantitative and qualitative immune response, all appeared to function at an adequate level in these HIV-infected individuals. First, a rapid booster (memory) response could be identified in most HIV-infected individuals and all healthy controls. Second, an immunoglobulin class switch from predominantly IgM to IgG antibodies was equally present in healthy controls and HIV-infected individuals. Third, a significant increase in antibody avidity after repeated vaccination, a process of clonal selection of the most specific antibody producing cells, was present in both HIV-infected individuals and healthy controls. Although all these processes are considered to be dependent on the number (and functioning) of CD4+ T-cells, only the class switch showed a statistically significant correlation with the number of CD4+ T-cells at the time of vaccination in the present study. Overall, the magnitude of the booster response was less in HIV-infected patients as compared to healthy controls but independent of the CD4+ T-cell count.

Several studies have shown that the immunological impairment caused by HIV is only partially reversed by antiretroviral treatment, even with a full recovery of the CD4+ T-cell count. [10-13, 20] The severity of this immunological 'scar' has been linked to numerous factors. The depth of the pre-treatment CD4+ T-cell nadir, thymic function and/or age, genetic factors, baseline viral load, the presence of HIV-RNA and the number of CD28 expressing CD4+ (central memory) T-cells, an essential co-factor for T-cell activation by stimuli such as a vaccine antigen, have all been implicated among others. [21-32] Currently, several studies support initiating HAART at higher CD4+ T-cell counts to prevent HIV-related morbidity and mortality, associated with ongoing viral replication and loss of immunological function. [33-38]

The present study is unique in that it contains a long-term follow up of patients with a quite severe pre-HAART immunodeficiency: 63% of the patients had a nadir CD4+ T-cell count of less than 200 and one third even less than 100 cells per microliter. The fact that a proper response upon a T-cell-dependent neo-antigen could be mounted despite a low CD4+ T-cell nadir underscores the complex but resilient nature of the immune system.

Vaccination studies in HIV-infected patients, like our own study, typically have a relatively small sample size and a limited immunological evaluation of the subjects, impairing the power to detect factors associated with a poor vaccination outcome. (Partial) immune recovery in the HAART era has been described for many vaccines including the influenza vaccine. [39-46] The most extensively studied vaccine in HIV-infected individuals is probably the hepatitis B virus (HBV) vaccine. HIV-infected individuals show a poor response upon the standard HBV vaccination (protection rates of 18-56%, even in the HAART era, contrasting with >90% in healthy controls). Clinical factors such as male gender, high body mass index and a detectable viral load, and immunological parameters

such as a low number of CD4+ T-cells or CD4+ memory cells and a high number of activated (CD38/HLA-DR positive) CD8+ cells have been associated with a worse HBV vaccination outcome. [29, 44-46] Even though the number of patients with a detectable viral load was very low in the present study and the majority of these patients only showed low level viremia, the presence of HIV-RNA in the plasma was associated with lower post vaccination titers upon rabies vaccination, possibly through immune activation.

Only a few studies report on rabies vaccination in HIV-infected patients: a poor antibody response on both pre- and post-exposure immunization schedules has been described in HIV-infected individuals with low CD4+ T-cell counts. [47-51] Guidelines consider rabies vaccination safe for this patient group and advise to measure the effect of the immunization, which consists of 3 vaccinations administered within four weeks. [52] The necessity of booster vaccinations for long term protection, especially for immunocompromised patients, is underscored by the present study.

The relatively high prevaccination concentrations of IgG anti-rabies in the HIV-infected individuals might be caused by cross-reactivity of antibodies against HIV gp120 with a rabies glycoprotein, which share a structurally and functionally related site. [53] The height of the prevaccination titers in HIV-infected patients in the present study did not affect the antibody responses upon subsequent primary and booster rabies vaccination.

In conclusion, the antibody response upon vaccination with a neo-antigen such as the rabies vaccine is impaired in HAART treated HIV-infected patients despite quantitative CD4+ T-cell recovery. However, functionally and clinically important aspects of the adaptive immunity such as the booster response, class switch and avidity maturation upon booster vaccination do seem to be operational and protective anti-rabies titers were induced in all subjects after only two vaccinations. These findings have important consequences for other vaccination strategies and vaccines, including HIV vaccines.

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Chapter | 7

Vaccination of HIV-infected adults

HIV therapy. 2009;3:565-72.

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1 **ABSTRACT**

2
3 Vaccination has been shown to be one of the most powerful tools to decrease morbidity
4 and mortality caused by numerous infectious diseases. The risk of (complications of)
5 some vaccine preventable diseases is higher in HIV-infected individuals, underscoring
6 the importance of vaccination in these patients. However, the response upon vaccination
7 is generally impaired and shorter lasting in HIV-infected individuals, especially in those
8 with low CD4 T-lymphocyte counts and detectable HIV-RNA, as compared to healthy
9 controls. Even in patients successfully treated with anti-retroviral treatment, an impaired
10 immune response may persist despite normalization of the CD4-cell count.

11 Caution with live attenuated vaccines is warranted in HIV-infected individuals with
12 low CD4 T-lymphocyte counts. Decisions about administering a live attenuated vaccine
13 should be made after weighing the risks and benefits on an individual basis. In this article
14 we review the immunology of vaccination in HIV-infected individuals, as well as the most
15 relevant caveats of vaccination in this patient group. The currently available information
16 about the 2009 influenza A/H1N1 monovalent vaccine is reviewed.

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1 INTRODUCTION

2
3 Preventing or mitigating disease by vaccination is one of the most (cost-)effective interven-
4 tions in medicine. [1] Routine vaccination programs for adults, that typically include
5 booster vaccinations every decade against diphtheria, tetanus, pertusis and polio, also
6 apply to HIV-infected adults. [2] In addition, influenza, pneumococcal, hepatitis A virus
7 and hepatitis B virus vaccines, are indicated in (most) HIV-infected adults. [3-5] The
8 effectiveness and safety of vaccines that prevent travel associated infections might be lower
9 in HIV-infected individuals as compared to healthy individuals. [6]

10 Numerous clinical and immunological factors have been correlated with an impaired
11 immune response upon vaccination; most notably a low CD4 count and a detectable
12 HIV-RNA at the time of vaccination. [7-13] Most clinical and immunological vaccination
13 studies in HIV-infected individuals, including our own studies, are relatively small in
14 sample size and rely on surrogate parameters, such as antibody titers, as primary outcome.
15 This review summarizes the general principles of vaccination of HIV-infected adults,
16 with the currently registered vaccines (thus excluding e.g. HIV vaccines and other vaccines
17 in development) and refers to relevant practical guidelines.

20 GENERAL CONSIDERATIONS

22 The immunology of vaccination and HIV

23 Although readily quantified by measuring the number of CD4+ T-lymphocytes, the im-
24 munodeficiency that defines HIV/AIDS is more complex than just a lack of periferal
25 T-cells. [14-15] The HIV induced depletion of CD4+ T-lymphocytes from its natural
26 reservoirs and the subsequent translocation of antigens from the gut sets of a complex
27 disruption of humoral and cellular immunity and lymphoid tissues. [16-19] Immune
28 activation and inflammation are important components of this process, that becomes
29 visible in lymph nodes. Lymph nodes in chronic HIV-infection are filled with activated
30 T-and B-cells, in part reactive against HIV, disrupting both architecture and function of
31 the lymphoid tissue. [20-21] The actual immune response upon vaccination takes place
32 in these hyperplastic lymph nodes, after the vaccine antigens have been transported by
33 antigen presenting cells from the vaccination site to the draining lymph node. In these
34 lymph nodes follicular dendritic cells capture and retain the antigen, presenting it to
35 antigen-specific B-cells that subsequently undergo clonal expansion, forming germinal
36 centers in which HIV is also abundantly present. With the help of follicular B-helper
37 T-cells (T_{FH}-cells) memory B-cells are formed, that facilitate a booster response upon
38 a second encounter with the identical antigen. [22] Although relatively little is known
39 about these specific processes in HIV-infected individuals, the final outcome is quite clear:

1 responses upon both T-cell-independent and T-cell-dependent antigens are impaired. In
 2 general, patients with a CD4 cell count less than 200 cells / μ l are considered severely im-
 3 munocompromised, those with CD4 cell counts in between 200 and 500 cells moderately
 4 and those with a CD4 cell count > 500 are considered to have a near normal immunity.

5 Antiretroviral treatment has brought a tremendous shift in the morbidity of HIV-
 6 infected individuals; opportunistic diseases have become exceedingly rare in those who
 7 receive medical care, even if the CD4 cell number does not fully recover. [23-25] This
 8 is a clear sign that HIV-treatment is followed by functional immune recovery. There are
 9 however many studies that show that this immune recovery is not complete and that an
 10 'immunological scar' remains, even after many years of successful HIV-treatment. [26-
 11 28] As illustrated in Figure 1, the typical response upon vaccination in HIV-infected
 12 individuals is impaired both in magnitude and longevity as compared to healthy controls.
 13 [29] Clinical failure of vaccination has been described. [30-31] Whether or not an earlier
 14 initiation of ART, at higher CD4-cell counts, might prevent immunological damage and
 15 thus aid to limit this scar is currently unknown. [32-34] The clinical importance of this
 16 immunologic scar might be limited. We found that many aspects of the T-cell-dependent
 17 immune responses upon a T-cell-dependent neo-antigen were functional in a group of
 18 HIV-infected individuals who had reached extremely low CD4 nadir counts in the pre-
 19 ART era but who subsequently reconstituted after the initiation of HAART. A booster
 20 response, class switch and affinity maturation were observed, as proof of the functionality
 21 of the newly formed CD4+ T-lymphocytes in these patients. [35]

22 Detectable HIV-RNA is a factor, independent of the CD4 cell count, that has been
 23 associated with impaired responses upon vaccination in several studies. [12,13] The
 24 underlying mechanism might be ongoing immune activation and inflammation or HIV-
 25 induced apoptosis of activated T-cells involved in the immune response upon the vaccine
 26 antigen. [36]

27 Almost all vaccination studies in HIV-infected individuals revert to surrogate pa-
 28 rameters such as antibody levels or specific T-cell responses to measure the quantitative
 29 response upon vaccination, since clinical endpoints (such as pneumonia in pneumococcal
 30 vaccine studies) are infrequent and only large field studies can determine an effect on mor-
 31 bidity and mortality. [37] Antibody levels correlate well with clinical protection (at least
 32 in healthy subjects). The antibody level that is considered protective is usually identical
 33 in healthy controls and immunocompromised patients such as HIV-infected individuals.

34 35 Optimizing the efficacy of vaccination

36 Vaccination should preferably be postponed in patients with a severe immunodeficiency (a
 37 CD4 count of less than 200 cells per μ l) if immune reconstitution and viral suppression is
 38 to be expected within a reasonable period of time. Patients treated with steroids for PCP
 39 should preferably wait for at least three months before receiving any vaccinations. Seasonal

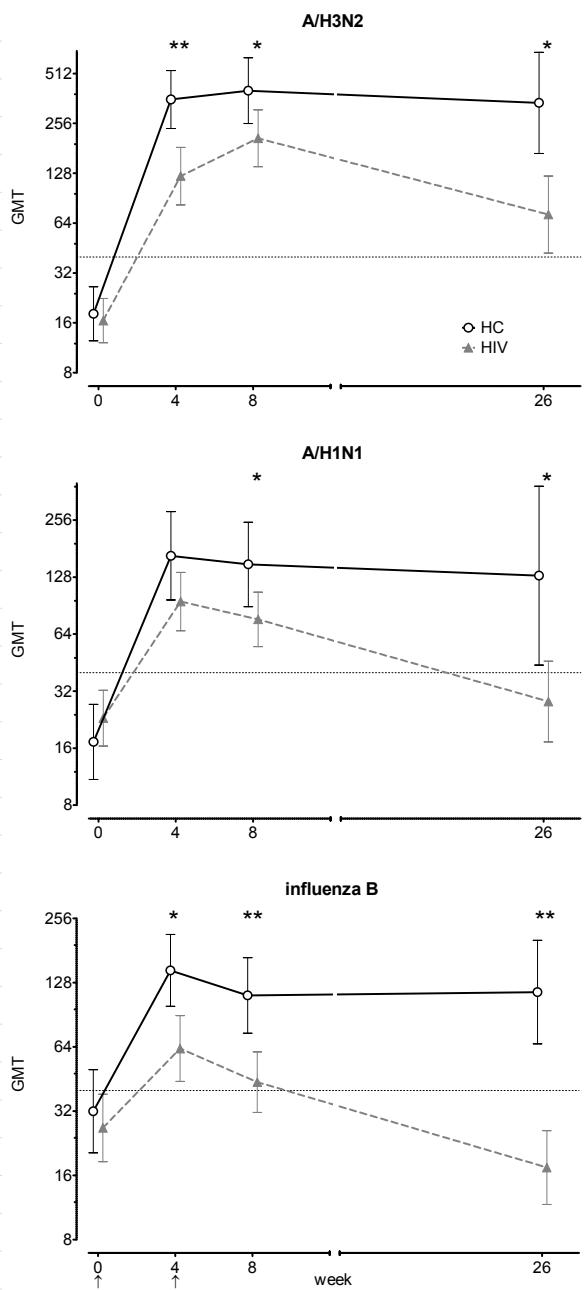


Figure 1. Twenty six weeks follow up of geometric mean titers (GMTs) of anti-influenza A/H3N2, A/H1N1 and influenza B antibodies upon influenza vaccination at week 0 and 4 for HIV-infected individuals (HIV, n=80, median CD4 T-cell count 463 cells/ μ l (IQR 293–415); 83% treated with ART, 74% undetectable (<40 c/ml) HIV-RNA) and healthy controls (HC, n=41). Represented are the pooled data from subjects who received intradermal and intramuscular vaccinations (seasonal vaccine 2005–2006). [29] Arrows indicate timing of vaccinations; * p<0.05; ** p<0.001.

vaccines or travel related vaccines should be administered when indicated, with some restrictions for live attenuated micro-organism and knowing that the immune response might be sub optimal. Determining post vaccination titers (e.g. after HAV and HBV vaccination) in HIV-infected adults identifies those who are unprotected upon vaccination and might subsequently benefit from alternative measures to protect them from disease.

Since the antibody response upon vaccination can be short lived, earlier re-vaccination might be necessary in HIV-infected individuals as compared to healthy controls.

Many strategies have been explored to optimize the response upon vaccination in immunocompromised patients. These strategies include higher vaccine doses, increased vaccination frequency, different formulations, addition of adjuvants, or different routes of administration (e.g. mucosal or intradermal). [29, 38-40] None of these strategies has been proven powerful enough to replace the routine practice.

Adverse reactions

Local and non serious adverse reactions, such as pain, myalgia and fever, do occur after vaccination in a frequency comparable to that in healthy controls. Local skin reactions upon intradermal vaccination occur less frequent in immunocompromised patients than in healthy controls. [29] It is unknown if severe but rare side effects, such as Guillain-Barré Syndrome after influenza vaccination, occur disproportionably more often in HIV-infected individuals, who are already prone for this condition. [41-42]

Some level of immune activation is necessary to mount an immune response upon vaccination. [22] This immune activation is usually mild, but might be accompanied by a transient rise of HIV-RNA, which most likely represents a transient increase of the residual viremia and not an overall increase of viable virus. [43-44] The impact of vaccination on the natural course of HIV-infection is likely to be marginal and not clinically relevant.

Live vaccines

As a general rule, live attenuated vaccines are contra-indicated in patients with an immunodeficiency, since the vaccine strain could be pathogenic and cause a variant of the disease it should prevent, if the immunity fails to neutralize the vaccine strain. Reports of disease caused by attenuated vaccine strains are rare, with the exception of lymphadenitis and other complications caused by the *M. tuberculosis*-vaccine strain *Mycobacterium bovis* BCG. [45-50]

Three vaccines (Yellow Fever (YF); measles, mumps and rubella (MMR) and varicella zoster virus (VZV)) are generally considered safe if the CD4-positive lymphocyte count is higher than 200 cells/ μ L. [3-4] A recent study showed that YF vaccination was safe and effective in HIV-infected adults with a median CD4-count higher than 500 cells/ μ L. Although this study included a few patients with a CD4-positive lymphocyte count of less than 200 cells, it obviously was not powered to detect even a 1000-fold increased risk of

the rare but severe complications of YF vaccination. [51] The indication for Yellow fever vaccination should carefully be made: for visitors to non-endemic countries a vaccination weaver could be preferred, while for persons visiting endemic YF area's the vaccine risks might be outweighed by the risks of contracting the disease.

Drug interactions

Vaccines are not dependent on (hepatic) metabolism or renal clearance and pharmacokinetic interactions are not an issue in the vaccination of HIV-infected individuals. There are however some (potential) interactions that deserve consideration.

For some flavi-viruses, including West Nile virus and Yellow Fever, a worse outcome has been described in patients with a dysfunctional or absent CCR5 receptor. [52-53] Therefore, the use of the Yellow Fever vaccine might not be safe in patients treated with CCR5 inhibitors, such as maraviroc and vicriviroc, as recently hypothesized. [54-57] The use of (pegylated-)interferon in patients co-infected with hepatitis C might prevent the necessary replication of a live vaccine virus strain, and thus prohibit an adequate immune response. However, data that support these hypotheses are lacking.

GUIDELINES FOR CLINICAL PRACTICE

Table 1 summarizes vaccine characteristics and efficacy data of commonly used vaccines in HIV-infected adults. [3,36,58] Practical guidelines for the immunization of HIV-infected adults are widely available online and should be integrated in the complex care of HIV-infected individuals. [2-6; 59] Adherence to these guidelines often is suboptimal. [60] Vaccination status (for e.g. HBV) and indications (for e.g. influenza) should explicitly be mentioned in the communication with the primary health care providers and patients.

THE 2009 INFLUENZA A/H1N1 MONOVALENT VACCINE

The introduction of an antigenically and genetically new human influenza A/H1N1 virus in April 2009 poses many new challenges. [61] This virus is a neo-antigen for the majority of the population, although there are clear indications of cross-reactive antibodies found in those who were infected with the 1918 influenza A/H1N1 and its daughter viruses. Some clinical protection is found in elderly (>60 years of age) who have been in contact with related influenza A/H1N1 viruses that circulated until many decades ago. [62-65] The T-cell-dependent neo-antigen vaccine was expected to generate a relatively weak and short-lived antibody responses, especially in individuals with a compromised T-cell immunity, such as (untreated) HIV-infected individuals with a CD4-positive lymphocyte

Table 1. Characteristics of vaccines and vaccine response in HIV-infected individuals.

vaccine	type (antigen type, vaccination route)	Incidence or morbidity of disease ‡	Sero-response ‡‡	remarks
Diphtheria	2 (toxoid, im)	=	=/↓	Secondary (booster) response impaired with CD4 count < 300
Haemophilus influenzae serotype B	2 (conjugated, im)	↑	↓	Most studies done with children
Hepatitis A	2 (inactivated, im)	↑	↓	Response rates correlate with CD4 count
Hepatitis B	2 (surface antigen, im)	↑	↓	Response rates correlate with CD4 count
Human papilloma virus	2 (viroosomal, im)	↑		'vaccine efficacy might be less' but no clinical trials yet
Influenza (seasonal)	2 (split or sub-unit, im)	=/↑	↓	Response reduced with CD4 count < 200
	3 (intranasal)	=/↑	=	Contraindicated; no prolonged viral shedding; seroresponse comparable to placebo in HIV and HCs †
MMR	3 (im)	↑ (measles)	=/↓	Durability of response may be reduced
Meningococcus	1 (PS, im)	=	↓	Limited data; durability of response may be reduced
	2 (conjugated, im)	=		No published data
Pneumococcus	1 (PS, im)	↑	=/↓	Shorter lasting protection due to lower peak antibody titers postvaccination; increased incidence of pneumonia in vaccinated adults (but decreased all cause mortality)
	2 (conjugated, im)	↑	↓	Limited data; prime (conjugate)-boost (polysaccharide) strategies have been explored
Poliomyelitis	2 (inactivated (IPV), im)	=	=/↓	Secondary (booster) response impaired with CD4 count < 300
	3 (OPV, oral)	=		Most studies done with children; contraindicated
Rabies	2 (inactivated, im)	=	↓	Shorter lasting protection due to lower peak antibody titers postvaccination
Smallpox	3 (vaccinia, intradermal)	↑		contraindicated
Tetanus	2 (toxoid, im)	=	=/↓	tetanus immunogenic (but less than HCs)
Typhoid fever	1 (PS, im)	↑	↓	Response rates correlate with CD4 count
	3 (Ty21a, oral)	↑		Contraindicated
Tuberculosis	3 (M. bovis BCG, id)	↑		Contraindicated
Varicella zoster virus	3 (Oka strains, im)	↑	=	Limited data; may be used with CD4 > 200
Yellow fever	3 (17D strain, im)	=	↓	May be used with CD4 > 200; faster decline of neutralizing antibodies; caution in patients treated with CCR5 inhibitors

Vaccine type:

1: T-cell-independent;

2: T-cell-dependent;

3: live attenuated (*generally contra-indicated*)

Im: intramuscular; PS: polysaccharide; id: intradermal; HCs: healthy controls

‡ More (↑), equal (=) or less (↓) as compared with healthy controls

‡‡ Better (↑), equal (=) or worse (↓) as compared with healthy controls

† serum response might not be adequate to monitor the immune response upon this vaccine

7__

8__ count less than 200 cells/ μ L. HIV-infected individuals with adequate immune restoration

9__ upon treatment with antiretroviral therapy responded just as well as healthy controls upon

10__ primary vaccination with a T-cell-dependent neo-antigen. [35]

11__ Three kinds of (monovalent) vaccines are being produced against influenza A/H1N1

12__ 2009, none of which include antigens that protect against seasonal influenza. [66] The

13__ unadjuvanted subunit vaccine and the live-attenuated virus vaccine are being produced

14__ with similar methods as the seasonal influenza vaccines, the latter being contraindicated

15__ in HIV-infected individuals. To increase the antibody response upon vaccination, an adju-

16__ vanted monovalent vaccine is also being developed. Repeated vaccination ≥ 3 weeks after

17__ primary vaccination is advised for all three vaccines. Although an interval shorter than

18__ three months is too short to generate a true booster response, repeated vaccination will

19__ lead to higher titers as is shown in figure 1 (for the seasonal influenza vaccine). There are

20__ no data yet about the antibody response upon the 2009 influenza A/H1N1 monovalent

21__ vaccine in immunocompromised individuals.

22__ HIV-infected individuals aged 25 – 64 years are among the groups indicated for vacci-

23__ nation with the 2009 influenza A/H1N1 monovalent vaccine according to the Centers for

24__ Disease Control and Prevention (CDC). [66] Reduced dose intradermal (unadjuvanted)

25__ seasonal influenza vaccination was as immunogenic as full dose intramuscular vaccination

26__ in HIV-infected adults. [29] This strategy, although not tested with this neo antigen,

27__ might be helpful in times of vaccine shortages.

28__

29__

30__ CONCLUSIONS

31__

32__ Most vaccines can be administered safely and effectively in HIV-infected individuals when

33__ certain precautions are taken. Complications from live attenuated vaccines, although rare,

34__ do occur in immunocompromised patients. Often safe alternatives for protection are

35__ available and individual vaccine policy should be based on a personal risk-benefit analysis.

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Summary and general discussion

Summary

Thesis

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1 INTRODUCTION

2
3 Patients with an impaired immunity, either due to the use of immunosuppressive medica-
4 tion, chronic HIV infection or a hematologic stem cell transplantation, are at a greater risk
5 of developing (opportunistic) infections. [1-4] The consequences of infections often are
6 more severe in immunocompromised patients as compared to healthy individuals. [5-8]
7 Both pneumococcal and influenza infections are associated with greater morbidity and
8 mortality in immunocompromised patients. [9-12] The impact of these two infections
9 can be reduced by vaccinating these patient groups, as advised by many international
10 guidelines. [13,14] However, the response upon vaccination is typically reduced in these
11 patients, due to the impaired immunity. [15,16]

12
13 In order to study different parts of the immune system we investigated the response upon
14 T-cell-independent (pneumococcal polysaccharide), T-cell-dependent (influenza) and
15 T-cell-dependent neo antigen (rabies) vaccines in several types of immunocompromised
16 patients.

17 Several aspects of the immune response have been evaluated.

20 SUMMARY

21
22 Chapter 1 and 2 resulted from the 'vaccination Response in ImmunoCompromised Hosts
23 1 (RICH1)' study, conducted in 2003/04; chapters 3, 4 and 5 resulted from the RICH2
24 study, conducted in 2005/06. The first three chapters of this thesis report on the results of
25 studies conducted in patients treated with relatively new immune modulating medication.

26
27 In *Chapter 1* the effect of anti-TNF with or without other immunosuppressive medica-
28 tion on the antibody response upon the 23 valent pneumococcal polysaccharide (PPS23)
29 vaccine is described. The antigens included in this vaccine initiate a T-cell-independent
30 immune response, although partial and non-specific dependency of T-cells has been de-
31 scribed for some of the antigens included in the vaccine. A true T-cell response, however,
32 (including the forming of memory cells) is not induced. In a mixed population of patients
33 with rheumatic diseases and inflammatory bowel disease we found that the combination
34 of methotrexate with anti-TNF was a potent inhibitor of the immune response. This
35 synergy between methotrexate and anti-TNF was also observed in several clinical studies
36 on the treatment of RA. [17] However, the underlying pathophysiological pathway has
37 not been elucidated.

38 *Chapter 2* describes the response upon the T-cell-dependent influenza vaccine in the
39 same study population as described in chapter 1. The surplus of infections with intracellu-

lar pathogens (such as mycobacteriae) seen in patients treated with anti-TNF, is suggestive of an impaired T-cell mediated immunity. We demonstrated that anti-TNF modestly but significantly inhibits the response upon the T-cell-dependent influenza vaccine. Unlike the response upon the T-cell-independent PPS23 vaccine, the combination of methotrexate with anti-TNF did not further enhance the impaired immune response upon influenza vaccination as compared to anti-TNF alone.

The results from the RICH1 study show that anti-TNF inhibits cellular immunity. Only when anti-TNF is combined with methotrexate, there is an additional inhibition of the T-cell-independent response. The synergy of anti-TNF and methotrexate is unique for the T-cell-independent immunity and not present in the T-cell-dependent immune response. The synergistic effect of anti-TNF and methotrexate thus most likely interferes with specific B-cell processes such as B-cell-receptor-antigen binding and signalling, processes that have been shown to be TNF-dependent. This finding shows insight into the mechanism of the synergy between these two drugs in the treatment of RA. The synergy observed in therapeutic trials might thus very well be attributed to the effect on the T-cell-independent (humoral) immunity. Inhibition of humoral immunity is a relatively novel concept in the treatment of RA, although 'old' drugs like sulfasalazine (and its metabolites) also have been shown to inhibit B-cell immunity.

In the *Appendix to chapter 1 and 2* additional data from the RICH1 study are shown, such as data from the week 8 follow up and the fact that the antibody response upon the T-cell-independent pneumococcal polysaccharides and the T-cell-dependent influenza hemagglutinin are independent of each other, representing distinct immunological pathways as illustrated in Figure 1.

In *Chapter 3* we evaluated the response upon influenza vaccination in RA patients treated with the specific anti-B-cell therapy, rituximab. Rituximab is now a common treatment option in patients failing anti-TNF therapy. In 2005, when this study was conducted, this still was an experimental treatment for RA patients, limiting the number of patients that could be included in this study group.

Nonetheless, we found a clear and significant inhibition of the antibody response 3 months after two rituximab infusions, with a low B-cell count at the time of vaccination. This study shows that a depleted B-cell number interferes with antibody production, which is the final common pathway of both T-cell-dependent and T-cell-independent immune responses.

Chapter 4 explores a possibility to enhance influenza vaccination outcomes in patients with an impaired immunity. On theoretical grounds we expected a more efficient antigen processing, possibly resulting in higher post vaccination antibody titers, when the influenza vaccine would be delivered intradermal as compared to the routine practice of intramuscular vaccination. In an animal model, intradermal vaccination gives rise to a

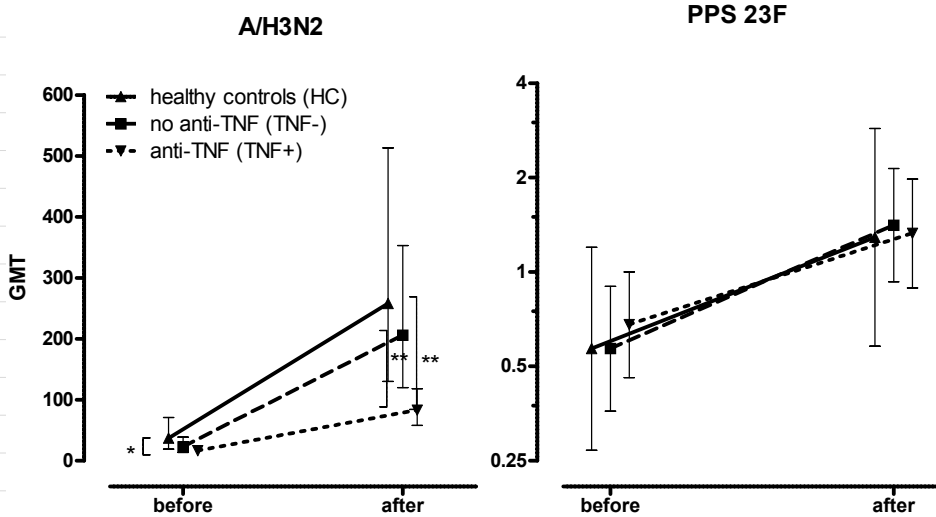


Figure 1. Antibody response (geometric mean titer) upon T-cell-dependent influenza (A/H3N2) and T-cell-independent pneumococcal polysaccharide (PPS9V) antigens within the same patient groups (RICH1 study) at t=0 (before) and t=4 weeks (after). * $p < 0.05$, ** $p \leq 0.001$.

faster and larger T-cell response in the draining lymph nodes as compared to traditional intramuscular vaccination. Recently, the superiority of intradermal vaccination has also been shown in humans.

We used a reduced dose (one fifth) of a trivalent subunit influenza vaccine for intradermal vaccination, in several groups of immunocompromised patients to exploit the superior immunological properties of the dermis. We found that a reduced dose intradermal vaccination elicited equal titers as compared to full dose intramuscular vaccination in healthy controls, rheumatologic patients treated with anti-TNF, HIV-infected individuals and hematologic stem cell transplant patients. A third study arm with the reduced dose (0.1 mL) intramuscular would have been a relevant comparator to truly compare the immunological properties of the dermis and the muscle. However, since there is a clear dose-response relation between the amount of vaccine administered and the subsequent antibody response, it was deemed unethical to administer a subtherapeutic dose intramuscular. The finding that a lower dose (when administered intradermal) can be as effective as regular full dose vaccination is both of immunological and clinical significance. Vaccine shortages often occur, illustrated by the fact that there was an actual influenza vaccine shortage in The Netherlands in the year this study was conducted (2005). Using vaccine antigens in a more efficient way could aid in resolving these shortages.

Local skin reactions upon intradermal vaccination proved to be predictive of the humoral immune response. Non-responders (a protective titer against zero out of three antigens) were identified by the absence of a skin reaction with a sensitivity of 83% but a specificity of only 57%. Responders (a protective titer against two or three out of three

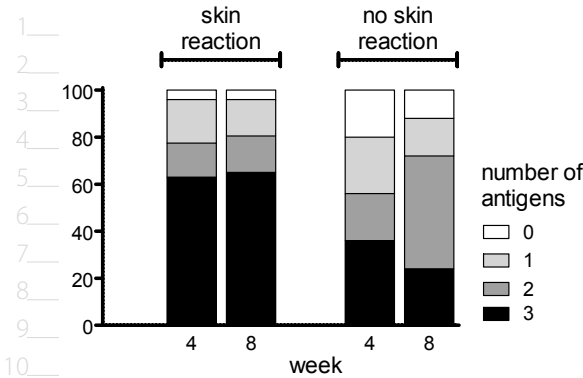


Figure 2. Number of antigens upon which a protective titer was formed four weeks after influenza vaccination (t=4) and four weeks after booster vaccination (t=8) in a group with and a group without a local skin reaction after intradermal vaccination (at t=0) in immunocompromised patients (n=52, RICH2 study).

antigens) were even less accurately identified by the occurrence of a skin reaction (sensitivity 60%, specificity 65%). Still, the relatively high sensitivity of the absence of a local skin reaction upon intradermal vaccination in immunocompromised patients could be useful in a clinical strategy to optimize the immune response: administering a second influenza vaccination after four weeks, only in those who do not develop a skin reaction upon intradermal vaccination. Repeated vaccination at week 4 increased the proportion of subjects that were protected to at least one or two of the vaccine antigens in this subgroup (Figure 2). In immunocompromised patients who did develop a skin reaction upon intradermal vaccination (52% of all immunocompromised patients), we found no additional gain in the percentage of patients with a protective titer after repeated vaccination at week 4 (Figure 2).

In the *Appendix to chapter 3 and 4* additional data of the RICH2 study are presented. These include the follow up up to week 26 and more detail on the intradermal vaccination and local adverse reactions.

In *Chapter 5* we investigated the forming of cross-reactive anti-influenza antibodies, a clinically desirable effect, upon influenza vaccination, in immunocompromised patients. Antibodies elicited by natural influenza infection or by vaccination show some cross-reactivity against different influenza strains. With increasing antigenic differences between different strains (the so called antigenic distance) the likelihood of cross-reactivity decreases. [18-19] This cross-reactivity is of clinical value in times when there is a mismatch between the influenza strain included in the vaccine and the circulating strain, a phenomenon known to occur with some regularity. It was shown before that elderly (≥ 75 years of age) are more at risk for influenza-infection in times of a strain mismatch, due to poorer cross-reactive properties of the antibodies elicited by vaccination.

1__ We re-tested the samples from the RICH2 study cohort (as described in chapter 4) for
 2__ the ability to react to the homologue (vaccine) strain and the cross-reactivity against drift
 3__ variants that appeared in the subsequent years. We found that cross-reactive titers waned
 4__ with increasing antigenic distance, as expected. The cross-reactive properties of antibodies
 5__ in healthy controls were similar as compared to those in HIV-infected individuals.

6__ This finding suggests that the mechanism that causes the impaired cross-reactivity in
 7__ the elderly differs from the immune defects found in HIV-infected individuals (with a
 8__ mean CD4-cell count of 440 cells / μ L).

9__

10__ In **Chapter 6** we describe a cohort of HIV-infected individuals, many of them survivors
 11__ of the pre-HAART era with CD4 T-lymphocyte nadir counts well below 200 cells per
 12__ μ L, before HAART was initiated, who were followed for almost a decade. These patients
 13__ were, after a mean of 41 months of HIV treatment, vaccinated twice with the rabies
 14__ vaccine, a T-cell-dependent neo-antigen, so that both the primary and booster response
 15__ could be investigated. At the time of vaccination a successful quantitative immune res-
 16__ toration was reached, in almost all patients, with a median CD4 T-lymphocyte count of
 17__ 537 (interquartile range 353-772) cells per μ L. Even though the overall post vaccination
 18__ titers were lower in HIV-infected individuals as compared to healthy controls, all T-cell-
 19__ dependent processes studied operated in a functional matter. A true booster response, an
 20__ IgM to IgG antibody class switch and avidity maturation were present in the HIV-infected
 21__ individuals, despite low T-lymphocyte numbers before initiating HAART. Five years after
 22__ vaccination, two thirds of the HIV infected patients still had antibody titers above the
 23__ protection threshold as defined by the World Health Organisation.

24__ This study suggests that the initiation of anti-retroviral therapy in HIV-infected indi-
 25__ viduals not only gives rise to a quantitative but, more importantly, to a qualitative immune
 26__ restoration. The complex interplay of antigen, antigen-presenting cells, cytokines and both
 27__ T- and B-lymphocytes, which has been shown to be disrupted in progressive HIV-disease,
 28__ appears to recover to a functional level after initiation of antiretroviral therapy. However,
 29__ even after more than 3 years of anti-retroviral therapy, this recovery is less than complete.

30__

31__ The general principles of vaccination in HIV-infected adults, illustrated with collated data
 32__ from the RICH2 study, are reviewed in **Chapter 7**. The use of live vaccines and medica-
 33__ tion interactions in HIV-infected individuals are being considered. A comment is made
 34__ on the new swine-origin influenza A/H1N1 (2009) vaccine.

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1 GENERAL DISCUSSION

3 The quantity and quality of the immune response in immunocompromised hosts

4 In the studies described in this thesis, we used vaccinations to measure several aspects of
5 the qualitative and quantitative immune response in patients with an impaired immunity.

6 The fact that anti-TNF inhibits the T-cell-dependent but not the T-cell-independent
7 response upon vaccination, clearly classifies anti-TNF as an inhibitor of the T-cell medi-
8 ated immune response, as would be expected. Exploring the mode of action of anti-TNF
9 is relevant for the understanding of the pathophysiology of e.g. rheumatoid arthritis and
10 subsequently for the development of even more specific immune modulating drugs. The
11 observations from vaccination studies add information to clinical studies, that show the
12 efficacy of these drugs as disease modifying drugs, without elucidating the mode of action.
13 [20-22] Our findings are in line with the surplus of opportunistic infections found in
14 patients treated with anti-TNF. [23-25] These infections are mainly caused by pathogens
15 such as intracellular bacteriae (mycobacteriae, Listeria, salmonellae) and yeasts (crypto-
16 cocci, histoplasma), which are associated with failing T-cell immunity. [26]

17 The finding that the combination of anti-TNF with methotrexate 'broadens' the im-
18 mune suppressive effect to include B-cell inhibition is a novel finding. Both rituximab
19 and the combination methotrexate and anti-TNF inhibit B-cell responses. However, their
20 mechanisms of action are quite different. Rituximab depletes CD20+ B-cells which are
21 the progenitors of antibody producing (plasma-)cells, and thus inhibits the final common
22 pathway of both T-cell-dependent and -independent immune responses. [27-30] The
23 combination of anti-TNF with methotrexate shows a synergistic inhibitory action only on
24 the T-cell-independent immune response, pinpointing its effect on 'early' B-cell processes
25 (before the final common pathway of T-cell-dependent and independent responses), such
26 as antigen-B-cell binding and the subsequent signalling. [31] This is in line with the
27 original concept of adding methotrexate to anti-TNF therapy to inhibit the forming
28 of antibodies directed against these drugs. Recent pathophysiological insights and drug
29 developments have concentrated on several other aspects of the B-cell involvement in
30 the (pathological) immune response. Blocking the B-cell activating factor belonging to
31 the tumor necrosis factor (TNF) family (BAFF), also known as B-lymphocyte stimulator
32 (BLyS), has been evaluated in the treatment of SLE. [32,33] BAFF and its receptor, trans-
33 membrane activator and calcium modulator ligand interactor (TACI), are upregulated
34 by ligation of toll-like receptors and by interferons, providing a link with pathogens or
35 vaccine antigens. BAFF deficient mice show immune responses comparable with those
36 found in patients treated with the combination of anti-TNF and methotrexate with a
37 greater impairment of T-cell-independent than T-cell-dependent responses upon vaccina-
38 tion. [34-36] Figure 3 is a schematic representation of the possible mechanisms by which
39 anti-TNF and rituximab can inhibit the immune response upon vaccination.

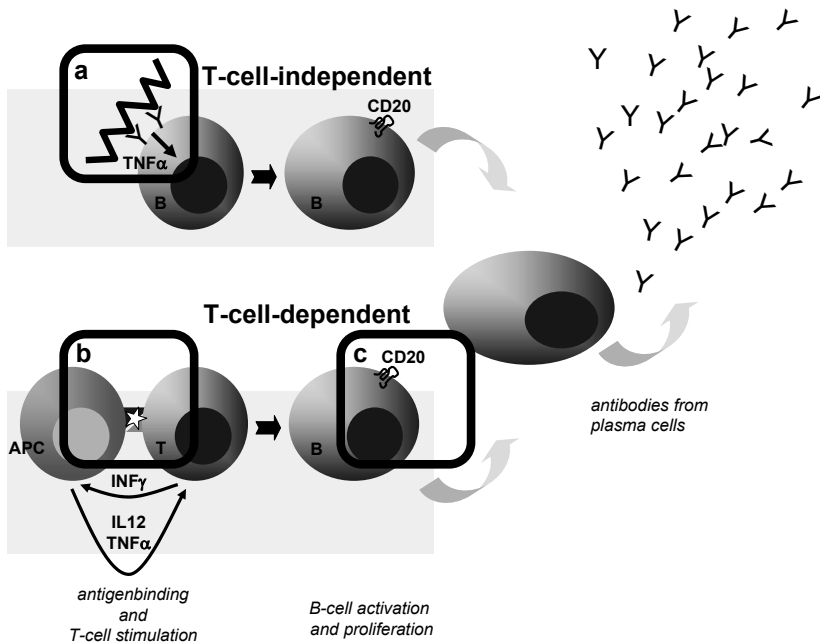


Figure 3. Schematic representation of the mode of action of methotrexate (MTX), anti-TNF and rituximab in their inhibition of the immune response upon vaccination.

a. The combination of MTX with anti-TNF specifically inhibits the T-cell-independent and not T-cell-dependent immune response. B-cell-Antigen binding and subsequent signalling have been shown to be TNF dependent. 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. (*chapter 1*)

b. The interaction of antigen-presenting cells and T-cells and the subsequent augmentation of the immune response upon a presented antigen is mediated in part through the action of TNF α . It is likely that anti-TNF inhibits this augmentation of the T-cell-dependent immune response (*chapter 2*).

c. Rituximab depletes CD20+ B-cells, either through complement-mediated cytotoxicity, antibody-dependent cell-mediated cytotoxicity or apoptotic or antiproliferative effects. This results in a severely hampered immune response upon neo-antigens (*chapter 3*). In this thesis we tested only the T-cell-dependent pathway. Since rituximab affects the common pathway of both T-cell-dependent and independent immune responses, it is highly likely that it also blocks the response upon a polysaccharide.

B: B-cell; T: T-cell; APC: antigen presenting cell

By evaluating several patients groups within the same vaccination protocol we found a clear hierarchy within the different groups, thus applying influenza vaccination as a tool to measure the severity of the immunodeficiency. This is a feasible strategy which could be employed in drug development trials, that evaluate the effect of immune modulating medication, to predict the extent of the immunosuppressive effect of these newer drugs. For drugs such as imatinib, a tyrosine kinase inhibitor, for example, the extent and the characteristics of the immunosuppressive effect are not yet clear. Years after registration,

with the growing use of this drug, case reports of tuberculosis possibly related to the use of this drug appear in the literature. [37-39] Evaluating the response upon (influenza) vaccination in patients on chronic imatinib therapy might shed some light on the extend of the immune deficiency. There are some clear disadvantages in the use of the influenza vaccine, such as the fact that the vaccine is updated annually; that global uniform outcome measurements are lacking and interference of possible confounders as described below. However, these disadvantages are outweighed by the fact that influenza vaccination can be used repeatedly, is inexpensive, easy to administer, safe, widely available and is indicated in immunocompromised patients. A T-cell-dependent neo-antigen, such as the rabies vaccine, allows for a more extensive evaluation of various aspects of the T-cell-dependent immune response, as shown in HIV-infected individuals (chapter 6). However, evaluating immune responses upon rabies vaccination is logistically more difficult and the individual responses upon the rabies vaccine are more variable as compared to the response upon influenza vaccination.

Additional determinants of the immune response

Age

Overall the antibody response diminishes with rising age. Although the three study cohorts described in this thesis (RICH1, RICH2 and rabies) were relatively young, this phenomenon was found in all three cohorts. Age proved to be a confounding factor in the rabies vaccination study, were the healthy controls were considerable younger than the HIV-infected individuals. The primary immune response, significantly impaired in HIV-infected individuals in a univariate analysis, proved to be statistically indistinguishable from that of healthy controls after adjustment for age.

The absolute number of naïve (CD4 and CD45RA positive) T-cells was inversely correlated with age (Figure 4). Diminishing thymus size and, secondary to this process, decreasing naïve T-cell numbers are physiologic aging processes. [40,41] Immune recovery after starting HAART in HIV-infected individuals is better at a younger age. [42,43] Many other factors have been described that might be correlated with this so called immunosenescence. [44] In the rabies study we found that higher age (in the setting of a low CD4 cell nadir before initiating HAART) was the most important factor, compromising the primary immune response upon vaccination with a T-cell-dependent neo-antigen, even after immune reconstitution as measured by a total CD4 cell count. In contrast, the booster response in these HIV-infected individuals was inhibited by the immunodeficiency and not by higher age. These data suggest that the age effect, found in numerous vaccination studies, most likely acts primarily through a compromised number of naïve T-cells, which are pivotal cells in the immune response upon a neo antigen. Age adjustments were made, where applicable in this thesis.

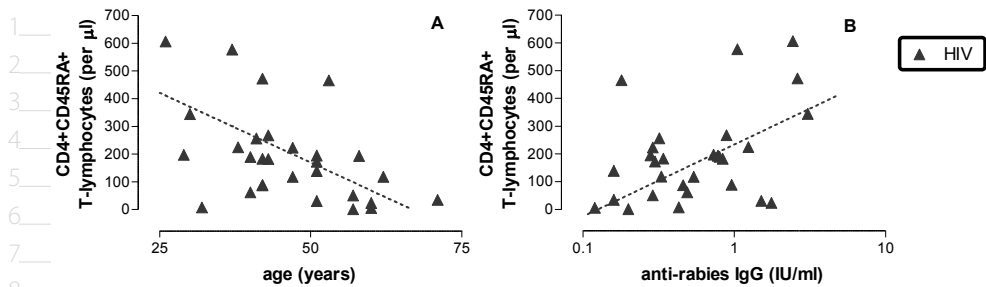


Figure 4. Correlation of the number of naïve (CD4+CD45RA+) T-lymphocytes with respectively age (panel A, Pearson correlation: $r = -0.55$, $p < 0.005$) and the IgG antibody response at week 4 after rabies vaccination (panel B, Pearson correlation: $r = 0.43$, $p < 0.05$; logarithmic transformed titers used for calculations).

Previous exposure

Previous exposure to the vaccine antigen is relevant in all vaccination trials. Prevacination titers are generally higher in patients who are vaccinated before. Furthermore, relatively high anti-influenza titers may persist for a long time after developing influenza, even if the disease course was mild and not recognized as 'the flu' by the individual. In the RICH1 study, for example, already 70% of the healthy controls had antibodies against the A/H3N2 vaccine strain, even though only one (6%) of them was vaccinated before (Figure 5). Repeated exposure to an identical antigen, will typically lead to an exponential titer increase (booster response) as shown in the rabies study. [chapter 6, this thesis] Previous influenza vaccination thus can act as an important confounding factor in determining vaccination outcomes and should be addressed in every influenza vaccination study. In Figure 6 the study outcomes (geometric mean anti-influenza titers) are represented based on whether the subject was vaccinated before (within pooled intramuscular and intradermal study groups). In some, but not all, experiments we found lower post vaccination titers in subjects with a history of previous vaccination, even when that vaccine antigen was identical to the one administered the year before. This counter intuitive outcome has been described before and was named 'the Hoskins' paradox' after the author of multiple influenza vaccination studies in the early 1970s. [45-47] Influenza vaccination is more complex than for instance rabies vaccination, since antigens are adapted on an annual basis, to match circulating strains. As shown in chapter 5 significant cross-reactivity does occur between related strains. Two processes might play a role in the paradoxical lower post vaccination titers in previously vaccinated subjects: 1. neutralisation of vaccine antigen (or the forming of immune complexes) by circulating antibodies and 2. a phenomenon known as the 'original antigenic sin' which means that a related antigen will boost the response upon the original antigen, as opposed to the newly administered antigen, when there is enough resemblance. This latter process may play a role in the findings of the RICH1 study (since higher prevaccination titers per se were not associated with lower post vaccination titers in previously vaccinated subjects, which is not in line with the first

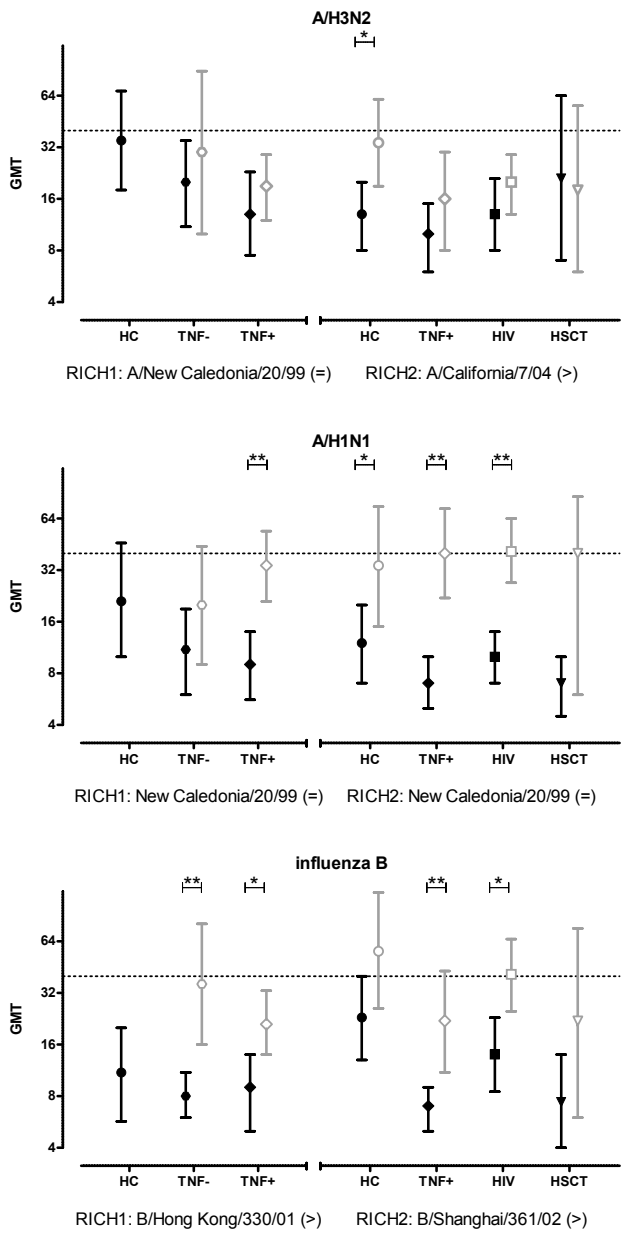


Figure 5. Prevaccination geometric mean titers against influenza A/H3N2, A/H1N1 and influenza B based on whether patients were previously vaccinated or not (pooled data from intramuscular and intradermal vaccination route, RICH2 study). Titers from previously vaccinated subjects also represent vaccination titers one year after influenza vaccination. Closed black symbols: not previously vaccinated; open grey symbols: previously vaccinated.

* $p < 0.05$; ** $p \leq 0.001$, comparisons only made within each study group.

(>) vaccine antigen different from antigen included in vaccine from previous season;

(=) vaccine antigen identical to antigen included in vaccine from previous season.

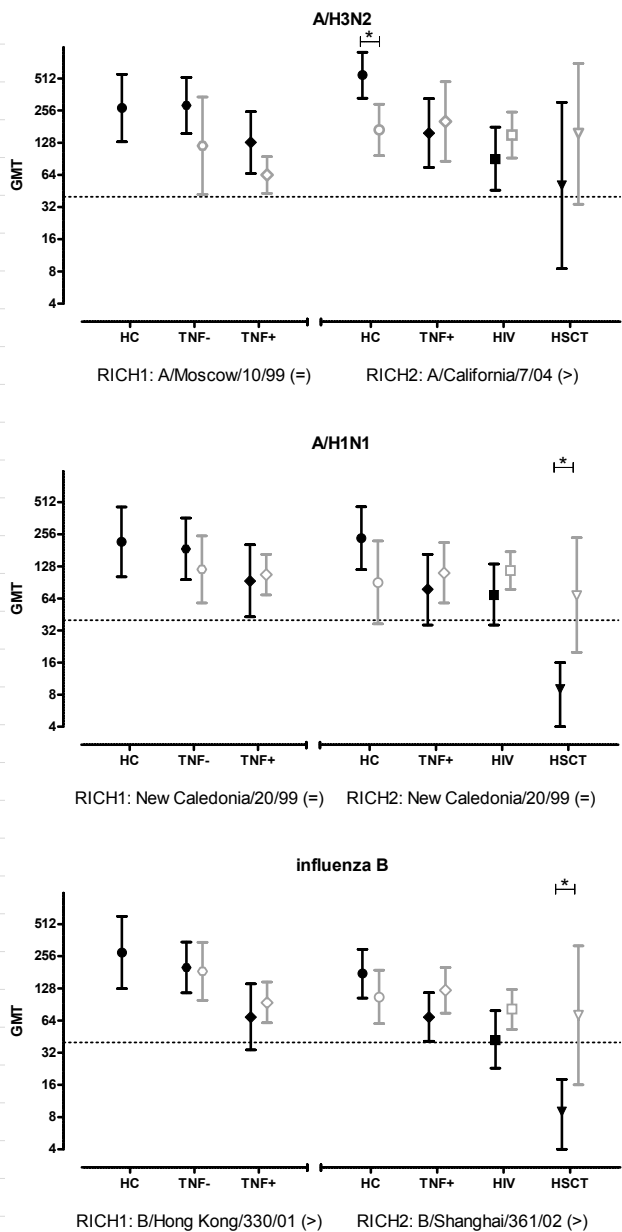


Figure 6. Postvaccination geometric mean titers against influenza A/H3N2, A/H1N1 and influenza B based on whether patients were previously vaccinated or not (pooled data from intramuscular and intradermal vaccination route, RICH2 study).

Closed black symbols: not previously vaccinated; open grey symbols: previously vaccinated.

* $p < 0.05$; ** $p \leq 0.001$, comparisons only made within each study group.

(>) vaccine antigen different from antigen included in vaccine from previous season;

(=) vaccine antigen identical to antigen included in vaccine from previous season.

1 explanation). The amount of vaccine used or the vaccination route did not influence the
2 magnitude of this effect in the RICH2 study.

3 In the studies presented in this thesis adjustments (for either previous vaccination;
4 the prevaccination titer or the interaction product of these two factors) were done where
5 applicable.

7 *Gender and other factors*

8 Gender has been reported in some vaccination studies as a relevant factor for which
9 adjustment was necessary. Both male and female gender have been correlated with better
10 outcomes in different studies. [48, 49] In none of our studies, gender proved to be a
11 relevant factor of influence on the antibody response. Figure 7 shows the vaccination
12 results from the RICH1 and RICH2 study (geometric mean titers against influenza A/
13 H3N2) divided by gender instead of immunization route.

14 Many other factors have been implied as variables that influence the outcome upon vac-
15 cination: among them the site of vaccine administration (e.g. deltoid vs. gluteal muscle),
16 body weight, smoking, blood (ABO) group and zidovudine, statin or cotrimoxazole use.
17 [50,51] These factors were not evaluated in our vaccination trials, although the route and
18 place of vaccination were fixed and statin use was rare. The (permuted-block) random-
19 ization procedures used, make it unlikely that any of these factors acted as significant
20 confounding factors.

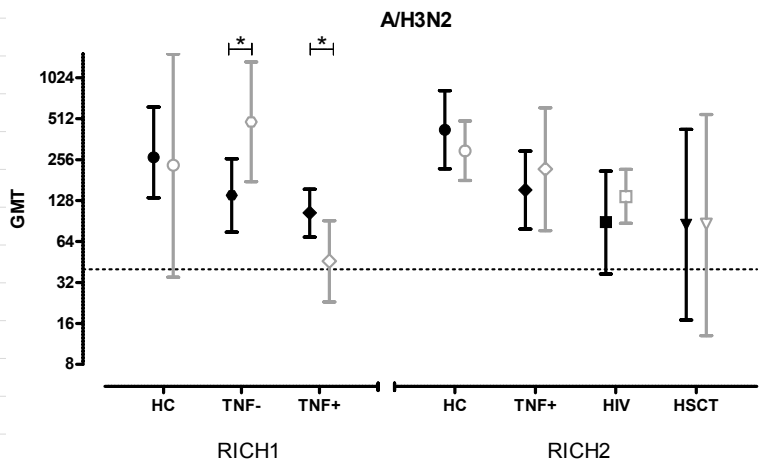


Figure 7. Postvaccination geometric mean titers against influenza A/H3N2 based on gender (pooled data from intramuscular and intradermal vaccination route, RICH2 study).

Closed black symbols: female; open grey symbols: male.

* $p < 0.05$, comparisons only made within each study group.

1__ The immunology of antibody production

2__ Two additional factors are important to realize when interpreting the data from our studies.
 3__ First, all outcome measurements (mainly antibody titers) used in this thesis are surrogate
 4__ parameters. True vaccine efficacy is established in large trials, to be able to determine a
 5__ protective effect on morbidity or mortality. [52] However, the study outcomes used in this
 6__ thesis have been correlated with clinical efficacy and geometric mean titers are suitable for
 7__ the comparison of groups, irrespective of the clinical effect. [53-55]

8__ Second, measuring antibody responses as a readout of the immune response, as was
 9__ done in the studies presented in this thesis, is a simplification of the immunological real-
 10__ ity. The immune response is far more complex than only antibody production, involving
 11__ many aspects of both innate and adaptive immunity, including cellular responses. In
 12__ between the administration of the vaccine and measuring the antibody level four weeks
 13__ later is an 'immunological black box'. Both primary and secondary immune responses
 14__ take place in lymphoid tissue and the physiology of this response upon vaccination is
 15__ reasonably well described in healthy subjects. B-cell activation upon antigen binding will
 16__ upregulate CCR7, a molecule that will drive antigen-specific B-cells to the outer T-cell
 17__ zone of lymphoid tissues. Antigen-specific B-cells are captured and retained by follicular
 18__ dendritic cells (FDCs) that, in cooperation with follicular T-cells, facilitate massive clonal
 19__ proliferation. The class switch from IgM to IgG, IgA or IgE secreting plasma cells and
 20__ affinity maturation will take place in the germinal centers (GC) that are formed upon
 21__ this proliferation. In a high turnover state the B-cell with the highest affinity for the
 22__ vaccine antigen will take that antigen from the FDCs, process this antigen and undergo
 23__ subsequent T-cell help for proliferation. Less specific cells will degrade in this process.
 24__ This process, also known as the somatic hypermutation process, will drive the response
 25__ towards the most specific antibody producing cells. In healthy subjects, the forming of a
 26__ GC reaction takes about 2 weeks, negative feedback starts within 3-6 weeks, thus peak
 27__ IgG levels can be found 4-6 weeks after primary vaccination. Although direct in situ
 28__ measurements were not conducted in the studies presented in this thesis and B- and T-cell
 29__ numbers were only determined in peripheral blood, our studies allow for some specula-
 30__ tions on what happens in 'the immunological black box' as described above. The rabies
 31__ vaccination study (chapter 6, this thesis) illustrates that GC forming takes place in both
 32__ healthy controls and HIV-infected individuals. In HIV-infected individuals the lymph
 33__ node architecture is largely destroyed by the immune activation that characterizes chronic
 34__ HIV-infection. Most HIV-infected individuals who participated in this study started with
 35__ HAART as soon as this therapy became available, the majority of these patients had CD4
 36__ counts below 200 cells/ μ L at that time point. From historical vaccination studies we know
 37__ that the T-cell-dependent immune response is severely compromised in patients with such
 38__ a severe immune deficiency. [56,57] The fact that all processes of the T-cell mediated
 39__ immune response (including class switch and affinity maturation) seem to be intact in the

study cohort described in chapter 6 is a strong indicator that the initiation of HAART leads to both quantitative and qualitative immune reconstitution. However, a minority of the HIV-infected individuals and none of the healthy controls showed a delayed response upon primary rabies vaccination (data not shown), which might be a clue that GC forming in HIV-infected individuals is indeed less efficient.

Whereas in HIV-infection the number of CD4⁺ T-lymphocytes is decreased and the architecture of the lymph nodes disrupted, patients treated with anti-TNF have a different kind of immune deficiency. TNF α is a versatile cytokine with many functions, one of them being a crucial cytokine in augmenting the Th1-type (pro-inflammatory) immune response. Antigen presenting cells (APCs) secrete TNF α upon antigen binding, which attracts and stimulates T-cells that produce interferon gamma, which in turn stimulates the APCs. B-cell proliferation is dependent on this process. Antagonizing TNF likely interferes with the augmentation of the immune response, thus impairing T-cell-dependent immune responses.

The positive correlation between (the absence of) a local inflammatory response upon intradermal vaccination and the (lack of a) subsequent immune response might be a reflection of the physiology of the immune response upon vaccination. Immature dendritic cells internalize vaccine antigens and are subsequently activated by the local inflammation, which is classically caused by the vaccine adjuvants. The absence of a local inflammatory response can thus contribute to an impaired immune response. The fact that local inflammatory reactions in general are largely TNF-alpha driven possibly contributes to the inhibitory effect of anti-TNF on the immune response upon vaccination.

Longevity of the immune response in immunocompromised hosts

The longevity is a third aspect of the immune response in immunocompromised patients, besides the quantity and quality, which is often reported as being inferior to that of healthy controls. [58] Follicular B-helper (CXCR5⁺) T-cells (T_{FH}-cells) are thought to be the pivotal cells for the development of memory B-cells. Although CXCR5 can not act as a co-receptor for HIV cell entry, the dependency of T-cells for the forming of B-cell memory, combined with the known T-cell dysfunction in HIV might provide a model for the shorter lasting immunity found in HIV-infected individuals. [59-61] Another explanation might be the impaired B-cell function described in HIV-infected individuals, which is already apparent directly after primary infection. Circulating memory B cells are severely reduced in the peripheral blood of (both acute and chronically) HIV-1-infected patients, which impairs maintenance of long-term serologic immunity to HIV-1-unrelated antigens. This defect appears not to be restored upon HIV treatment. [62,63]

In the rabies vaccination study we were able to follow up all patients five years after vaccination. The anti-rabies IgG titers in HIV-infected subjects, coming from lower

(geometric mean) peak values four weeks after booster vaccination, remained above the protection threshold for a shorter period as compared to healthy controls. Still, the majority of HIV-infected individuals had a titer above the protection threshold five years after vaccination. Subjects with the highest titers four weeks after the booster vaccination were more likely to be protected five years later.

Patients on anti-TNF (and rituximab) therapy, who received influenza vaccination before, showed remarkably low prevaccination titers (as rest-titer one year after the previous vaccination), even if the vaccine antigen had remained unchanged, indicating a short lasting response in these patients (Figure 5). The low anti-influenza titers 1 year after vaccination in patients treated with anti-TNF indicate that antagonizing TNF interacts with the forming of memory B- and T-cell clones. This is conceivable since TNF α is, as stated before, a critical cytokine in augmenting the T-cell-dependent immune response, both on the site of antigen delivery as in de GC.

In the RICH2 study we analyzed samples taken six months after vaccination. The titers at this time-point remained relatively stable and well above the protection threshold in healthy controls but not in HIV-infected individuals. This is a strong indication that the decreased longevity of the immune response upon vaccination in HIV-infected individuals is not merely the effect of lower peak values, but that titers actually decrease faster. This suggests that long-lived plasma cells also show a decreased survival in HIV-infected individuals. The 6 month follow up data from the RICH2 study can not be translated to the common influenza vaccination practice, because our subjects were vaccinated twice; the immune response after a single vaccination might well be more short lived.

These data indicate that not only the quantity of the immune response is negatively influenced by an impaired immunity, but also that the duration of the time above the protection threshold is shorter. In chapter 5 we show that the cross-reactivity of anti-influenza antigens in HIV-infected individuals is comparable to that found in healthy controls, which might be of clinical benefit for protection against influenza drift variants.

A note should be made that both memory B- and T-cells might persist for decades, even in the absence of an antigen. These cells will promptly respond on repeated contact with an antigen, providing an almost instantaneous (booster) immune response. The absence of antibodies is thus not per se synonymous with the absence of immunity. [64]

Optimizing the immune response

As stated earlier, optimizing the immune response is most crucial for those who have a compromised response upon vaccination and the highest risk of disease. It seems prudent to postpone vaccination in HIV-infected individuals until the immunity has recovered and HIV-RNA suppression has fully occurred. Detectable HIV-RNA is a factor, independent of the CD4 cell count, that has been associated with impaired responses upon vaccination in several studies. The underlying mechanism might be ongoing immune activation

1 and inflammation or HIV-induced apoptosis of activated T-cells involved in the immune
 2 response upon the vaccine antigen. [65] In the influenza study (chapter 4) the presence of
 3 detectable plasma HIV-RNA levels was not associated with lower postvaccination titers.
 4 In the rabies study only a limited number of subjects with a detectable HIV load was
 5 included, these subjects did however show a trend for lower postvaccination titers.

6 In the rabies study (chapter 6) we vaccinated subjects who were treated with antiretro-
 7 viral treatment for a mean of 3.4 years. Whether or not immune recovery is an ongoing
 8 process at that time point, is unknown. It is however likely that some 'immunological
 9 scars' remain, even after a decade or more of effective antiretroviral treatment. Initiating
 10 anti-retroviral treatment at higher CD4 cell numbers, which has recently been shown to
 11 reduce the chance of AIDS and mortality, might aid in limiting the size of this scar. [66-68]

12 Many strategies have been explored in immunocompromised patients in order to op-
 13 timize vaccination outcomes, including increased dosage, multiple dose vaccination, the
 14 use of vaccine adjuvant, immunostimulant patches and more efficient routes of vaccine
 15 delivery. [69-72]

16
 17 We explored the latter, since the dermis is one of the best equipped organs for recognizing
 18 and processing antigens. The abundance of antigen presenting cells in the dermis, facili-
 19 tates the efficient transport of (larger quantities) of antigen to the germinal centers where
 20 the actual immune response is formed. Both in HIV-infected individuals and in patients
 21 treated with anti-TNF this strategy might bypass the immune defects (as described above)
 22 that impair the response upon vaccination in these patients. [73-76] In healthy subjects
 23 some vaccines (especially adjuvants containing vaccines) give rise to a severe local inflam-
 24 matory response with granuloma forming or even necrosis, which limits the possibilities
 25 to use the dermis for routine vaccination in healthy subjects.

26 Although in the RICH2 study intradermal vaccination did not lead to higher post
 27 vaccination titers, similar titers were found with a fraction of the dose administered intra-
 28 muscular. This finding, combined with the fact that there is a dose-response relationship
 29 between the amount of vaccine given and the height of the post vaccination antibody
 30 titer strongly suggests that intradermal vaccination is more efficient. Formal proof of this
 31 theory was cumulated from both animal and human studies. [77-79] It is suggested that
 32 lower vaccine doses may result in more competition for antigen in the GCs, resulting in
 33 lower antibody titers that express a higher affinity. [80] Although we did not measure
 34 affinity, we did determine cross-reactivity of anti-influenza antibodies. More specific (high
 35 affinity) antibodies would be expected to show less cross-reactivity. The route (and thus
 36 the amount of vaccine given) did not influence the cross-reactivity in the RICH2 study;
 37 but the amount of antigen that is presented in the lymph node might be equal after
 38 low dose intradermal and regular dose intramuscular vaccination (due to more efficient
 39 antigen capture and presentation).

1 In both the RICH1 and 2 study we administered a second influenza vaccine dose at
 2 week four. The timing of this 'booster' vaccination might be too early to expect a true
 3 (logarithmic) booster response and was chosen mainly for practical reasons. On average,
 4 titers continued to rise after the second vaccination (Figure 1, appendix 1; Figure 3 ap-
 5 pendix 2), although this further increase was modest and led only to a small number of
 6 individuals who 'seroconverted' only after this second vaccination. If we would have used
 7 the absence of a local skin reaction upon intradermal vaccination as the criterion to select
 8 non-responders, we would have selected the proportion (48%) that would benefit the
 9 most from a booster vaccination.

12 CONCLUSIONS AND RECOMMENDATIONS

14 Immune modulating drugs such as anti-TNF and rituximab inhibit the immune response
 15 upon vaccination. The inhibition of the antibody response upon influenza vaccination
 16 gives an indication of the severity of the (T-cell-dependent) immunodeficiency, irrespec-
 17 tive of its mechanism. Influenza vaccination should be incorporated in industry initiated
 18 trials assessing immune modulating drugs, since it helps in quantifying the immune sup-
 19 pressive potential of these drugs.

21 An impaired immunity strengthens, not weakens, the indication for vaccination, with the
 22 exemption of live attenuated vaccines. All vaccinations should preferably be administered
 23 before initiating or intensifying immunosuppressive therapy.

24 The T-cell-dependent properties of an immune response can be restored in HIV-
 25 infected individuals treated with HAART, even when the nadir CD4 positive T-cell count
 26 was once below 200 cells / μL . Vaccines in HIV-infected individuals should preferably be
 27 administered after immune restoration and full suppression of HIV-RNA.

29 Intradermal influenza vaccination offers some advantages in patients with impaired im-
 30 munity: 80% of the vaccine dose is spared without compromising the effect of the vaccine.
 31 The absence of a local skin reaction can be used to identify non-responders upon vaccina-
 32 tion. These non-responders might benefit from other strategies to prevent influenza (such
 33 as repeated vaccination or prophylactic or pre-emptive use of antiviral medication).

35 Vaccination policy should be an integrated part of the care for patients with an impaired
 36 immunity or those about to receive immunosuppressive drugs. Adapting vaccination
 37 strategies as suggested in this thesis, might be of great clinical benefit to those with a
 38 compromised immunity.

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Samenvatting

**Nederlandstalige samenvatting
(voor niet-medici)**

Proefschrift

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1 INLEIDING

2

3 In dit proefschrift worden vaccinatiestudies bij mensen met een verminderde afweer
4 beschreven. Het doel van deze studies is niet het beschrijven van de afweerreactie *sec.* De
5 vaccinatie studies zijn gebruikt als meetinstrument, om uitspraken te doen over het soort
6 en de ernst van de verschillende afweerstoornissen. Voor nieuwe afweeronderdrukkende
7 medicatie kunnen we uitspraken doen over waar deze middelen in de afweer aangrijpen en
8 hoe sterk dit afweerremmende effect is. Bij mensen geïnfecteerd met het humane immu-
9 nodeficiëntievirus (HIV), een virus dat juist CD4-positieve T-lymfocyten aantast, kunnen
10 we aan de hand van de reactie op vaccinatie uitspraken doen over allerlei onderdelen van
11 die (T-cel) afweer.

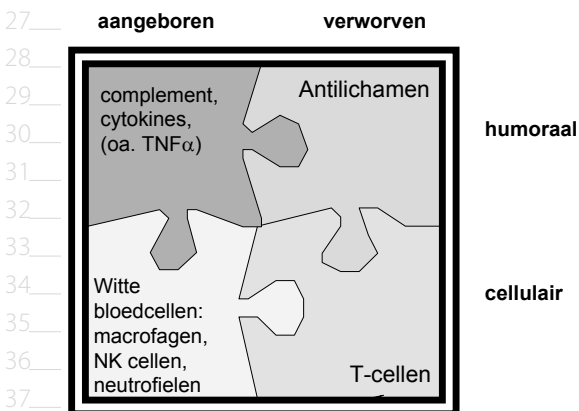
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13 De afweer

14 Onze afweer beschermt ons tegen invloeden van buiten, binnen de infectieziekten heten
15 deze schadelijke indringers als groep *pathogenen* (micro-organismen die ziekte kunnen
16 veroorzaken). Pathogenen worden onder andere onderverdeeld in virussen, bacteriën,
17 (gisten en) schimmels en parasieten.

18 De menselijke afweer heeft zich ontwikkeld tot een extreem geavanceerd apparaat
19 bestaande uit vele onderdelen. Binnen de mechanische barrières van de huid en de
20 slijmvliezen zijn er verschillende manieren om de afweer onder te verdelen. De afweer
21 laat zich bijvoorbeeld onderverdelen in een onderdeel bestaande uit cellen, de cellulaire
22 afweer, en een onderdeel bestaand uit moleculen (die vaak door de afweercellen worden
23 geproduceerd), de humorale afweer.

24 Een andere manier om de afweer in te delen is naar reactiesnelheid (en aanpassings-
25 vermogen). Een deel van de afweer is direct beschikbaar, behoeft geen aanpassing aan
26



38 **Figuur 1.** De afweer puzzel: schematische weergave van de verschillende onderdelen van de afweer en
39 de infecties waartegen die onderdelen ons beschermen.

1 een bepaalde indringer, maar is daarmee ook weinig specifiek (de aspecifieke, aangeboren
 2 afweer). Dit staat tegenover een deel van de afweer dat zich aanpast aan de indringer:
 3 afweer op maat (de specifieke, adaptieve afweer). Dit proces kost echter tijd, soms wel
 4 weken, wat veel te traag is voor veel infecties die binnen dagen of zelfs uren veel schade
 5 kunnen geven aan hun gastheer. Een voordeel van dit type afweer is wel dat er een ge-
 6 heugen wordt gevormd voor de specifieke pathogenen waarop ooit is gereageerd. Bij een
 7 hernieuwd contact met een identiek pathogeen kan er dan wel een snelle mobilisatie van
 8 de specifieke afweer plaatsvinden. Met name dit concept is van groot belang bij vaccinatie:
 9 het is (voor een aantal infectieziekten) mogelijk mensen levenslang te beschermen met
 10 een éénmalige vaccinatie(serie). Een schematische weergave van de afweer is afgebeeld
 11 in Figuur 1, waarbij de scheidslijnen tussen de verschillende onderdelen van de afweer
 12 overigens niet haarscherp zijn.

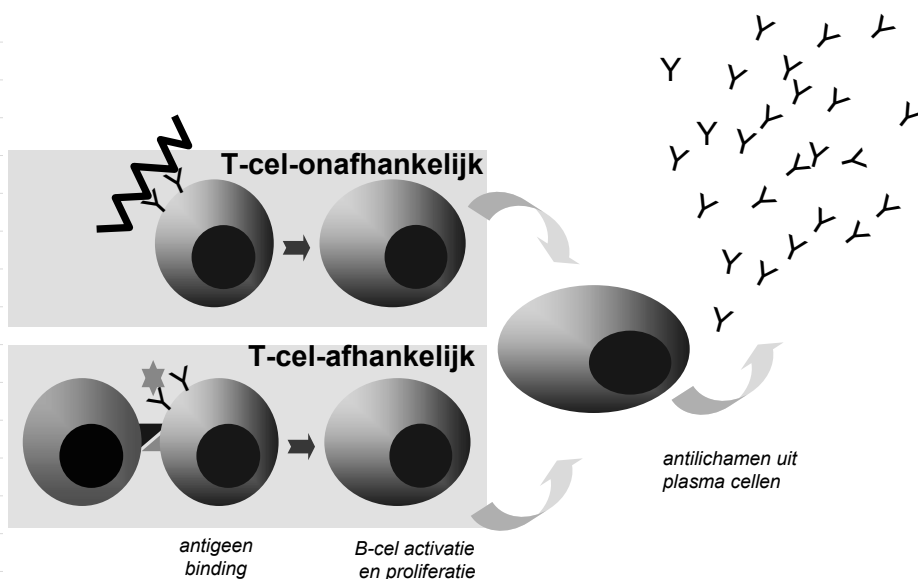
13 14 Vaccinatie

15 Het principe van vaccinatie is dat we afweer tegen een bepaald pathogeen oproepen, zonder
 16 dat we daarvoor die ziekte zelf hoeven door te maken. Dit kan bijvoorbeeld door alleen de
 17 meest afweerstimulerende onderdelen (zogenaamde antigenen) van een micro-organisme
 18 te gebruiken (en niet het gehele pathogeen dat ook in staat zou zijn de gastheer ziek te
 19 maken). Omdat hiervoor de specifieke (verworven) afweer wordt aangesproken, betreft
 20 het dan ook alleen afweer tegen deze geselecteerde micro-organismen. Het '23 valente'
 21 pneumokokkenvaccin, beschermt bijvoorbeeld niet tegen alle pneumokokken, maar al-
 22 leen tegen de 23 'serotypen' waarvan een stukje kapsel is opgenomen in het vaccin. De
 23 'griep prik' beschermt niet tegen alle denkbare virale luchtweg infecties, maar alleen tegen
 24 infecties met de specifieke influenza stammen die dat jaar in het vaccin zijn opgenomen.

25 De meeste vaccins werken beschermend door het oproepen van specifieke antistoffen.
 26 Antistoffen worden geproduceerd door plasmacellen, die weer voortkomen uit B-cellen.
 27 Bij T-cel-onafhankelijke vaccins kan het antigeen eigenhandig een B-cel aanzetten tot
 28 antistof productie tegen specifiek dat antigeen, het pneumokokkenpolysacharidevaccin is
 29 hier een voorbeeld van. De meeste vaccins, waaronder ook het influenza- en het rabiësvac-
 30 cin, zijn afhankelijk van de hulp van T-cellen om een B-cel (plasmacel) aan te zetten tot
 31 antistof productie (Figuur 2).

32 Bij sommige pathogenen lukt het om met een beperkt aantal (meestal T-cel-afhankeli-
 33 jke) vaccinaties levenslange bescherming te geven, bij andere vaccins (met name T-cel-
 34 onafhankelijke, zoals de polysacharidevaccins) is de duur van de bescherming korter. Er
 35 zijn nog steeds heel veel pathogenen (waaronder HIV) waarvoor het niet is gelukt een
 36 adequaat vaccin te ontwikkelen.

37
38
39



Figuur 2. T-cel-afhankelijke en T-cel-onafhankelijke immuunreactie na vaccinatie.

Afweerstörungen

Zo divers als onze afweer zelf is, zo divers is ook het spectrum van defecten in de afweer. In dit proefschrift is een aantal uiteenlopende, veel voorkomende, afweerstörungen naast elkaar gezet.

Er zijn ziekten die leiden tot een afweerstörung, zoals een infectie met HIV. Als HIV niet behandeld wordt, zal een specifiek onderdeel van de cellulaire afweer, het aantal CD4-positieve T-cellen, dalen en uiteindelijk zelfs geheel verdwijnen uit het bloed. Verder kan het een bewuste medische manipulatie zijn om de afweer (of althans een deel daarvan) te remmen. Dit gebeurt bij transplantaties, om te voorkomen dat het lichaam het transplantaat afstoot. Dit gebeurt ook bij zogenaamde auto-immuunziekten, ziekten zoals reumatoïde artritis (RA) of inflammatoire darmziekten (IBD), waarbij de eigen afweer ziekte veroorzaakt door onderdelen van het eigen lichaam abusievelijk aan te vallen. Auto-immuunziekten zijn daarmee de meest voorkomende reden voor het gebruik van afweereroderdrukkende medicatie: immuunsuppressiva. De laatste jaren zijn er belangrijke innovatieve ontwikkelingen, waarbij nieuwe immuunsuppressiva (zoals anti-TNF en rituximab) zeer actief blijken te zijn bij de behandeling van o.a. RA en IBD.

Vaccinatie bij mensen met een gestoorde afweer

Een afweerstörung betekend in de praktijk ook meestal dat de afweer minder goed reageert op vaccinatie (minder hoge antistof titers, kortere beschermingsduur). De kans dat vaccinatie dan niet tot bescherming voor die ziekte leidt neemt dan toe. In de praktijk wordt voor een aantal vaccins dan ook geadviseerd om te controleren of er een voldoende

1 afweerreactie is geweest na vaccinatie (en om, waar mogelijk, alternatieve maatregelen te
2 treffen als er onvolledige bescherming is).

3 Vaccinatie is over het algemeen niet schadelijk bij mensen met een verminderde afweer.
4 Alleen als er levend verzwakte vaccins worden gebruikt is er kans op ziekte veroorzaakt
5 door de vaccinstam. In dit proefschrift worden geen studies met levend verzwakte vaccins
6 beschreven.

7

8

9 **SAMENVATTING**

10

11 In **Hoofdstuk 1 en 2** worden de resultaten beschreven van een vaccinatie studie die in
12 2003/2004 werd uitgevoerd. We gebruikten twee verschillende soorten vaccins om van
13 2 verschillende kanten te kijken naar het effect van anti-TNF bij mensen met een auto-
14 immuunziekte.

15 Het pneumokokken(polysacharide)vaccin test de T-cel-onafhankelijke afweer (**hoofd-
16 stuk 1**). Het gebruik van anti-TNF alléén geeft geen relevante remming van deze afweer-
17 reactie. Als anti-TNF echter gecombineerd wordt met een ander immuunsuppressivum,
18 methotrexaat, wordt een sterke remming van de T-cel-onafhankelijke immuunrespons
19 gezien. De combinatie van deze twee middelen bleek eerder ook al synergistisch te zijn bij
20 de behandeling van RA.

21 In **hoofdstuk 2** laten we zien dat anti-TNF wel de T-cel-afhankelijke afweerreactie
22 remt. Het synergistische effect tussen anti-TNF en methotrexaat werd hier niet gezien.
23 Met deze bevindingen maken we het waarschijnlijk dat ook het therapeutische effect van
24 de combinatie anti-TNF en methotrexaat via het humorale (T-cel-onafhankelijke) pad
25 loopt.

26 In de **appendix bij hoofdstuk 1 en 2** wordt het vervolg na week 4 na vaccinatie ge-
27 toond als mede het gegeven dat de 2 verschillende vaccins gebruikt in deze studie ook
28 daadwerkelijk verschillende immunologische routes testen.

29 In **hoofdstuk 3** tonen we de reactie van RA patiënten, behandeld met rituximab, een
30 krachtig B-cel onderdrukkend middel, op het influenzavaccin. Alhoewel het om slechts 4
31 patiënten gaat is het overduidelijk dat rituximab een adequate antistofproductie blokkeert.

32 In **hoofdstuk 4** beschrijven we de studie waarin wordt onderzocht of de uitkomst van
33 influenzavaccinatie te verbeteren is bij verschillende groepen immuungecompromitteerde
34 patiënten. Vaccins worden normaliter toegediend in een spier. Dit heeft een logistieke
35 achtergrond, namelijk dat in de spier en halve milliliter vloeistof kan worden ingespoten
36 met een minimale kans op complicaties. Afweercellen zijn echter niet van nature aanwezig
37 in een spier en moeten worden toegevoerd via het bloed. De huid echter is doorregen
38 met een fijnmazig netwerk van afweercellen dat bedoeld is om antigenen vroegtijdig op
39 te sporen. De huid is daarmee een meer logische plaats om een vaccin toe te dienen, al is

1__ het maximale volume wat in de huid is toe te dienen maar ééntiende milliliter. Dit laatste
 2__ is een relatief nadeel, omdat een afweerreactie sterker is naarmate er meer antigeen wordt
 3__ gegeven.

4__ Toch bleek uit onze studie dat we met 5x minder vaccin intradermaal toegediend, we
 5__ een gelijkwaardige afweerreactie konden oproepen vergeleken met de normale dosis in
 6__ de spier, zowel bij de gezonde controles als bij de verschillende groepen patiënten met
 7__ een verminderde afweer. Deze strategie kan kostenbesparend zijn en helpen om zo veel
 8__ mogelijk mensen te beschermen als er vaccin te korten zijn (zoals het geval was voor het
 9__ influenza vaccin in het jaar dat we deze studie uitvoerde). Intradermale toediening bleek
 10__ in deze studie echter niet superieur aan intramusculaire vaccintoediening.

11__ Door verschillende groepen immuungecompromitteerden in één studie te testen kon
 12__ er een hiërarchie worden gemaakt van de ernst van de afweerstoonis binnen zo'n groep.

13__ Een nadeel van de vaccinatie in de huid is dat dit bij de meeste mensen een lokale
 14__ roodheid na vaccinatie geeft. Opvallend was dat deze lokale huidreactie ontbrak bij (83%)
 15__ van de immuungecompromitteerden die geen antistoffen produceerde op (alle 3 de stam-
 16__ men vertegenwoordigd in) het influenzavaccin. Daarmee is de afwezigheid van een vroege
 17__ lokale huidreactie in deze groep wel bruikbaar om mensen op te sporen die niet beschermd
 18__ zijn na vaccinatie, en dus mogelijk baat zouden kunnen hebben bij een tweede vaccinatie
 19__ (of andere maatregelen om griepinfectie te voorkomen).

20__ In de *appendix bij hoofdstuk 3 en 4* wordt onder andere het lange termijn beloop van
 21__ de antistoftiters getoond. We zien bij mensen die met HIV geïnfecteerd zijn de antistofti-
 22__ ter na vaccinatie sneller afnemen dan in gezonde controles.

23__ In *hoofdstuk 5* kijken we naar de vorming van kruisreagerende antistoffen. Hoewel
 24__ de antistof productie zeer specifiek is gericht tegen het vaccin-antigeen, zien we dat deze
 25__ antistoffen ook wel enige bescherming geven tegen antigenen die erg lijken op het vaccin-
 26__ antigeen. Hier hebben we gekeken naar de influenza-antigenen die in de jaren na onze
 27__ vaccinatie studie gingen circuleren. De antigene aanpassingen van deze virussen waren
 28__ niet heel groot, maar groot genoeg om de vaccinsamenstelling hierop aan te passen.

29__ Een eerdere studie liet zien dat ouderen slecht in staat waren kruisreagerende antistof-
 30__ fen te produceren. Daarmee kan een vaccin falen als de vaccinstam in een bepaald jaar
 31__ niet geheel overeen blijkt te komen met de 'epidemische stam' die uiteindelijk dat seizoen
 32__ circuleert. Zo'n 'mismatch' kan zo nu en dan optreden omdat de vaccinstammen ruime
 33__ tijd van te voren gekozen moeten worden. In de tussentijd kan een griepvirus zich verder
 34__ evolueren waardoor het niet meer (geheel) overeenkomt met de gekozen vaccinstam.

35__ Het onvermogen om breder kruisreagerende antistoffen te produceren, zoals gevonden
 36__ bij ouderen, werd niet gevonden bij mensen die geïnfecteerd zijn met HIV. Zij zullen,
 37__ ook als er een beperkte 'mismatch' is tussen de vaccinstam en het circulerende virus, wel
 38__ beschermd zijn tegen de griep.

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1__ In **hoofdstuk 6** vervolgen we een cohort van 30 HIV-geïnfekteerden over langere tijd.
 2__ Zij werden geïncubeerd in een rabiës (hondsdolheid) vaccinatie studie, nadat ze gemid-
 3__ deld 41 maanden behandeld waren met HIV-remmers. Velen van hen hadden een ernstig
 4__ verminderde afweer op het moment (rond 1996) dat zij met HIV-remmers begonnen.
 5__ Door de HIV-behandeling was bij iedereen het aantal CD4-positieve T-lymfocyten weer
 6__ tot normale waarden hersteld op het moment van vaccinatie. Door een neo-antigeen te
 7__ gebruiken (een antigeen dat het immuunstelsel niet eerder heeft gezien) konden we testen
 8__ of deze nieuw gevormde CD4-cellen ook functioneel in staat waren 'normaal' bij te dragen
 9__ aan de immuunrespons. We bekeken drie aspecten van de afweerreactie die afhankelijk
 10__ zijn van CD4-cellen. Het bleek dat al deze processen weliswaar weer functioneerde maar
 11__ altijd nog minder dan bij gezonde controles.

12__ Vijf jaar na de rabiës vaccinatie bleek tweederde van de HIV-geïnfekteerden nog steeds
 13__ beschermd tegen hondsdolheid.

14__ **Hoofdstuk 7** is een overzichtsartikel over vaccinaties bij mensen die geïnfecteerd zijn
 15__ met HIV. Het bevat onder andere de gecombineerde data van de studie beschreven in
 16__ hoofdstuk 4; een tabel met praktische adviezen en commentaar op de recente 'Mexicaanse
 17__ griep' vaccinatie campagne.

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20__ **DISCUSSIE**

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22__ De studies beschreven in dit proefschrift geven een beeld van verschillende aspecten van
 23__ de afweer bij een aantal groepen immuungecompromitteerden. Daarmee dragen deze
 24__ studies bij aan het begrip van het werkingsmechanisme van immuunsuppressiva en de
 25__ functionaliteit van de afweer bij mensen die geïnfecteerd zijn met HIV.

26__ Hoewel de studies voortkwamen uit praktische vragen van onszelf en andere klinici,
 27__ zijn er meer antwoorden gevonden dan alleen het antwoord op die vragen (bijvoorbeeld:
 28__ 'Is het zinvol om iemand die met anti-TNF behandeld wordt de grieprik te geven?').
 29__ Maar zoals aangegeven in één van de stellingen, het onderzoek roept ook weer veel vragen
 30__ op. Vragen die aanleiding geven tot verder onderzoek.

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Curriculum Vitae

CV

Proefschrift

L.B.S. Gelinck

*Dept. of Infectious Diseases, Leiden University Medical Center (LUMC), Leiden and
Dept. of MMI, Erasmus Medical Center, Rotterdam, The Netherlands*

1 CURRICULUM VITAE

2

3 Luc Ben Stefan Gelinck, geboren in Nijmegen op 30 april 1970, kreeg zijn eerste vac-
4 cinatie op 24 juli 1970 (D.K.T.P. I). In 1988 behaalde hij zijn atheneum-B diploma aan
5 het Mill Hill College in Goirle, waarna hij geneeskunde ging studeren in Leiden.

6 Gedreven door een bijzondere interesse in de immunologie deed hij zijn eerste onder-
7 zoek bij de afdeling reumatologie (Academisch Ziekenhuis Leiden) onder begeleiding van
8 dr. J.M.W. Hazes, voor het behalen van de docteraal bul in 1993.

9 Zijn co-schap interne geneeskunde liep hij in 1994 op de 'HIV/AIDS-afdeling' van het
10 Leyenburg Ziekenhuis, Den Haag, onder supervisie van dr. R.H. Kauffmann. Geïntrig-
11 eerd geraakt door de vele gezichten van HIV/AIDS, toen nog een onbehandelbare infectie,
12 nam hij in 1995 contact op met dr. F.P. Kroon om het laatste (facultatieve) deel van zijn
13 co-schappen aan HIV/AIDS-zorg te wijden. Dit co-schap werd, na het behalen van het
14 artsexamen in 1996, het jaar van vele kritische veranderingen rondom HIV-zorg, omgezet
15 in een aanstelling als arts-onderzoeker bij de afdeling infectieziekten van het Academisch
16 Ziekenhuis Leiden.

17 Na dit jaar keerde hij terug in het Leyenburg Ziekenhuis om zijn opleiding tot internist
18 te starten (opleider dr. J.C.M. van der Vijver), een opleiding die 6 jaar later in Leiden werd
19 afgerond (opleider prof. dr. A.E. Meinders). Weer een jaar later registreerde hij zich als
20 internist-infectioloog (opleider prof. dr. J.T. van Dissel). Tot 2007 bleef hij aan de afdeling
21 infectieziekten van het Leids Universitair Medisch Centrum verbonden om zich bezig te
22 houden met de academische trias (onderzoek, klinische werkzaamheden en onderwijs).
23 De onderzoeken beschreven in dit proefschrift zijn in die tijd zijn opgestart en uitgevoerd.

24 Vanaf 2007 is hij werkzaam bij de afdeling Medische Microbiologie en infectieziekten
25 van het ErasmusMC te Rotterdam. Zijn laatste vaccinatie (de 2^e mexicaanse griepvrij)
26 kreeg hij op 9 december 2009, de immuunrespons is onbekend.

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Publications

Publications

Thesis

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