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Vaccine-induced antibody responses as parameters of the influence of endogenous and environmental factors

H.van Loveren, J.G.C.van Amsterdam, R.J.Vandebriel,
T.G.Kimman, H.C.Rümke, P.S.Steerenberg, J.G.Vos

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RIVM, P.O. Box 1, 3720 BA Bilthoven, telephone: 31 - 30 - 274 91 11; telefax: 31 - 30 - 274 29 71

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Samenvatting

De beste manier om in het proefdier effecten op het immuunsysteem vast te stellen, is de bestudering van effecten op antigeen specifieke immuunresponsen, bijvoorbeeld na sensibilisatie met T-cel afhankelijke antigenen. Waarschijnlijk gaat dit ook op voor het testen van effecten in de bevolking. Om die reden is gesuggereerd antilichaam responsen na vaccinatie als uitleessysteem te gebruiken. Naast omgevingsfactoren worden vaccinatieresponsen beïnvloed door andere factoren. Eén factor die van grote invloed is, is het vaccin zelf en de procedure om te vaccineren. Daarnaast is de intrinsieke capaciteit van de recipiënt om te reageren van belang; dit wordt bepaald door genetische factoren en de leeftijd. Daarnaast zijn psychologische stress, voeding en ziekte (waaronder infectieziekten) van belang.

Het voorliggende rapport geeft een overzicht van de literatuur over invloeden op vaccinatieresponsen. Het blijkt dat voor wat betreft exogene factoren er duidelijk aanwijzingen zijn dat roken, voeding, psychologische stress en bepaalde infectieziekten een effect op vaccinatieresponsen hebben, maar dat het moeilijk is vast te stellen wat het relatieve belang ervan is. Bekend is dat genetische factoren sommige individuen voor sommige vaccins ongevoelig maken. Een algemene conclusie is dat in epidemiologische studies, waarbij nadelige effecten van blootstelling aan omgevingsfactoren op het immuunsysteem worden bestudeerd, en waarbij vaccinatietiters worden gebruikt, die additionele factoren in aanmerking genomen dienen te worden. Indien voor deze additionele factoren wordt gecorrigeerd, kan een studie waarbij associatie wordt gevonden van een verminderde vaccinatierespons met blootstelling aan een omgevingsfactor indiceren dat het immuunsysteem sub-optimaal functioneert. Het is niet waarschijnlijk dat een dergelijk effect zal inhouden dat bescherming waarvoor de vaccinatie was bedoeld wordt aangetast. Alleen in die gevallen waarbij individuen een matige respons vertonen, zouden nadelige effecten wellicht tot een klinische significante afname van bescherming kunnen leiden. Meer in het algemeen zou kunnen worden vastgesteld dat een afname in vaccinatierespons aan kan geven dat er een verminderde weerstand zou kunnen bestaan tegen pathogenen waartegen niet is gevaccineerd.

Summary

The most adequate way to assess effects of environmental exposures on the immune system using laboratory animals is to study effects on antigen-specific immune responses, such as after sensitization to T cell dependent antigens. Most likely, this also applies for testing effects in the human population. For this reason it has been suggested to utilize antibody responses to vaccination as readout. In addition to environmental factors, vaccination responses may be influenced by a variety of other factors. One factor is the vaccine itself, and the vaccination procedure. In addition, the intrinsic capacity of the recipient to respond to a vaccine is important, which is determined by genetic factors and age. Also psychological stress, nutrition, and (infectious) diseases are likely to have an impact.

The present report reviews the literature. It appears that with regard to exogenous factors, there is good evidence that smoking, food, psychological stress, and certain infectious diseases have an impact on vaccination titers, although it is difficult to state the magnitude. Genetic factors render certain individuals non-responsive to vaccination. In general, the conclusion is that in epidemiological studies of adverse effects of exposure to agents in the environment, in which vaccination titers are used, these additional factors need to be taken into consideration. Provided that these factors are corrected for, a study that shows an association of exposure to a given agent with diminished vaccination responses may indicate a suboptimal function of the immune system, and clinically relevant diminished immune response. It is quite unlikely that environmental exposures that affect responses to vaccination, may in fact abrogate protection to the specific pathogen for which vaccination was meant. Only in those cases, where individuals have a poor response to the vaccine, exogenous factors may perhaps have a clinically significant influence on resistance to the specific pathogen for which the vaccination was meant. An exposure-associated inhibition of a vaccination response may, however, signify a decreased host resistance to pathogens against which no vaccination had been performed.

1. Introduction

Antibody responses to vaccination are influenced by a variety of endogenous factors including genetics, gender, age, and exogenous factors such as stress, nutrition and infectious diseases. These factors need to be taken into consideration in clinical and epidemiological studies where the antibody response is the biomarker to be assessed, for example when one wants to assess in immunotoxicology investigations effects of exposure to environmental agents.

Studies in laboratory animals have shown that many environmental chemicals exert immunotoxic activity as indicated by altered immune functions, including effects on resistance to experimental infections (reviewed by IPCS, 1996). Effects of environmental exposures on immune functions have also been shown in humans (IPCS, 1996), yet, it is less well known whether immunotoxicity induced by environmental chemicals will have such severe consequences for resistance to infections. It has been suggested that where exposure to environmental immunotoxicants may induce subtler immunosuppression, consequences of this suppression may express themselves in increased incidences of common infections, like influenza and common cold (IPCS 1996).

In experimental studies in rodents, it has been shown that the antibody response to sheep erythrocytes is the most adequate indicator for immunotoxicity (Luster et al., 1992; 1993). This is due to the fact that the humoral immune response to sheep erythrocytes involves major components of the immune system, such as degradation of the erythrocytes by phagocytes, antigen presentation, cellular immune functions resulting in helper activity, and finally production of specific antibodies. In addition, it was shown that alterations in the response to sheep erythrocytes correlated well with resistance to experimental infectious agents in these animal studies (Luster et al., 1993).

This immune function test was also applied in non-human primates, i.e. exposure to PCBs of female rhesus monkeys (Macaca mulatta) reduced the antibody response to sheep erythrocytes, in conjunction with effects on several other immunologic parameters (Arnold et al., 1993).

For obvious reasons it is not possible to use antibody titers to sheep erythrocytes to study immunotoxicity in human populations. It was therefore suggested to establish effects of immunotoxic exposures on the specific immune response to infectious agents, i.e. to vaccines, instead (Yu et al., 1998; Van Loveren et al. 1999). Depending on the vaccine, major components of the immune system may be involved in the ultimate humoral response to the vaccine that may be induced. It is not possible for all vaccination responses to extrapolate the vigor of the antibody response with protection. Ideally, vaccination is performed in such a way that those variations in the response do not result in altered protection, albeit that protection is not always achieved in every individual vaccinated. Effects of exposure to immunotoxicants on vaccination titers, if and when they are observed, will therefore not necessarily indicate a decreased protection of individuals to the pathogen at which the vaccination is aimed. Rather, this may serve as a model of effects of exposures on immune responses to (other) infectious agents required for proper resistance to (other) infections.

2. Influence of environmental exposures on resistance to infectious diseases and vaccination titers

Patients suffering from immune deficiency develop more frequent, more severe, and often atypical infections, depending on the type of the deficiency. Complications of severe immunodeficiency include bacterial, viral, fungal, and parasitic infections. The respiratory tract is a primary target for infectious pathogens, especially in immunosuppressed patients. For instance, infectious complications have been commonly described in patients treated with various cytotoxic drugs for cancer treatment and with immunosuppressants, such as cyclosporin A, for the prevention of allograft rejection or the treatment of autoimmune disorders (Kim, 1989; Descotes and Vial, 1994).

Also, such consequences of environmental exposures have been documented in the literature. For instance, children born in between July 1978 and June 1987, to mothers that had been exposed to toxic levels of polychlorinated biphenyls (PCBs) and dibenzofurans (PCDFs) through consumption of contaminated rice bran oil in 1978-1979 showed higher frequencies of upper respiratory tract infections (Yu et al., 1998).

Exposure to organochlorines of Inuit mothers in Nanuvik (Arctic Quebec) through the food chain has been reported to increase the incidence of otitis media in their breast fed children (Dewailly, observations to be published). A relative risk factor of 9 in one year old infants in the highest tertile of exposure as compared with children in the lowest tertile was calculated.

An effect of background exposure to polychlorinated biphenyls and dioxins on the prevalence of otitis media was also reported in a group of toddlers in the Netherlands (Weisglass-Kuperus, observations to be published). In this study at 42 months of age an association with the cumulative exposure during these 42 months (odds ratio 3.58, with confidence limits of 1.04-12.28) was observed. In addition, an association of the prevalence of chickenpox with maternal exposure was observed with an odds ratio of 1.54 (and confidence limits of 1.01-2,35).

It is remarkable to note that, if vaccination titers are considered to be valuable for the detection of immunotoxicity in humans, the number of studies that have used this read out system is small. Even for controlled medicinal immunosuppression, for instance by immunosuppressants as cyclosporin, such studies are scarce. In a study of human antibody production as a response to treatment with murine monoclonal antibodies, decreased anti-mouse antibody production was shown after cyclosporin treatment (Weiden et al., 1994). After treatment for acute leukemia children had reduced antibody responses to DT-IPV vaccination (Van der Does et al., 1981). Also post transplant (organs, bone marrow) immune suppression has been shown to lead to a long period of hyporesponsiveness to vaccinations (Gerritsen 1994, Balloni 1999, Huzly 1997).

One study that does not involve humans, but in stead a semi-field study in seals, that included vaccination, was published by De Swart et al., 1995. These authors fed captive seals with herring from the Baltic Sea, or from the Atlantic Ocean. The relative contamination of the herring by polyhalogenated hydrocarbons, notably polychlorinated biphenyls, was 10 fold in the Baltic compared to the Atlantic herring. In seals that were fed with the Baltic herring, a significantly decreased specific antibody response to ovalbumin was observed.

In a study performed in 1976 by Reigert and Graber, the specific antibody response to tetanus toxoid was studied in 19 children, 12 of which were exposed to lead at concentrations that induced metabolic impairment. The antibody responses appeared unaffected. No other immune parameters were included in that study, so it is unclear whether immunotoxicity occurred in these children, albeit that lead certainly has been identified as an immunotoxicant (Lawrence, 1985).

One example of effects on antibody responses in humans is posed by smoking. Increased specific serum IgA and IgG responses to *Chlamydia pneumoniae* were observed by Von Hertzen et al. (1998a,b). Obviously, these responses were to the natural infection by the pathogen and not a vaccin. Hence, alterations in specific antibody titers, caused by smoking, may be a reflection of effects of the course of the infection and the subsequent antibody titers, rather than a reflection of a direct influence of smoking on the immune response to Chlamydia. However, in other studies, smoking has been shown to interact with specific antibody production. Smoking has been implicated in sub-optimal responses to vaccination with hepatitis B (Wood et al., 1993; Roome et al., 1993). In contrast, elevated antibody titers to influenza vaccination were also noted in smokers (Mancini et al., 1998).

A final example of studies on effects of environmental exposures on specific antibody titers after vaccination is the studies performed in the Netherlands by the group of Weisglas-Kuperus (Weisglas-Kuperus et al, 1995; Weisglas-Kuperus, to be published; Sas, to be published). The researchers observed lower antibody responses to measles and rubella in some breast-fed infants. At 42 months of age, there was a statistically significant negative correlation of antibody titers to measles vaccination with the exposure to polychlorinated biphenyls and dioxins as determined in cord blood (Spearman's Rho –0.20), and a statistically significant negative correlation of antibody titers to rubella with maternal exposure to these compounds (Spearman's Rho –0.21). However, after correction for gender, early feeding type (formula-fed or breast fed), duration of breast feeding during infancy, tobacco smoking by one or both of the parents, family history of atopy, and day care or nursery attendance, definitive conclusions could not be drawn since other confounders may have been relevant.

In a study performed by Termorshuizen et al. (1999), an association of season with specific antibody levels after hepatitis B vaccination was established in health care students. In the course of the vaccination procedure, involving multiple vaccinations, higher antibody titers were observed from the time of the second vaccination onward, when the first and second vaccination were applied in a winter period as compared to the summer. At the completion of the vaccination regimen, similar levels of antibodies were reached in both study groups. This finding was in accord with the working hypothesis of the authors, i.e. an immunosuppressive effect of ultraviolet radiation exposure expressed as diminished antibody titers after vaccination; ultraviolet radiation exposure obviously being highest in the summer. Yet, a definitive conclusion on the causal relationship cannot be drawn from this study, as other factors may have had an influence on the vaccination titers, for which no correction was performed.

3. Variability in vaccination titers due to the vaccination

A number of factors that related to the way vaccination is carried out determine the qualitative and quantitative immune response to the vaccine.

3.1 Number of vaccine doses (in the case of non-replicating vaccines)

In single individuals and in the population the (average) concentration of antibodies depends on the number of vaccine administrations. More vaccine generally gives higher antibody levels, as reviewed by Halsey and Galaska (1985), and thereafter confirmed in numerous studies (EPI, 1993).

3.2 Spacing of doses

In infant vaccination schedules that have longer intervals between doses, the post-vaccination antibody titers are usually higher than in short-spaced vaccination series. Short spaced vaccination series however induce protection earlier (Halsey and Galazka, 1985; Booy et al., 1992; EPI, 1993).

3.3 Vaccine concentration

Most vaccines available are formulated to contain an optimal concentration. Some vaccines result in a better priming and a higher antibody response when a higher dose is given (Salk et al., 1978; EPI, 1993). In practice this is be only relevant for vaccines that are available in a range of concentrations (depending on the indications), such as hepatitis A and hepatitis B vaccines.

3.4 Kinetics of the immune response

A first dose of an inactivated vaccine often does not induce a detectable antibody response, yet is active in priming B- and T-memory cells. There is a vigorous reponse to subsequent booster doses (secondary immune response). Peak levels of antibodies are found 1-3 months after the booster vaccination; then the levels decline. A next dose usually induces a peak response again, and the following decline will end at a higher base level.

Hence, the vaccine, vaccination route, and time point during or after completion of the vaccination procedure will have impacts on the vaccination titers. Therefore, if vaccination titers are used as a read out in epidemiological studies, it is of importance to standardize these.

4. Socio-geographic impact

In some vaccine studies using the same lots of vaccines and schedules the response in one group is higher than in the other group (e.g. in Turkish vs. Belgian infants, in Apeldoorn vs. Rotterdam infants), suggesting that genetic and/or environmental factors affect circulating antibody levels following immunization (Labadie et al., 1996; Hoppenbrouwers et al., 1999).

Similar differences have been found in measles antibody seroprevalences among immunized Inuit, Innu, and Caucasian subjects. Here too, the higher measles-seropositive rate found among native compared to non-native Canadian children may point at genetic as well as environmental factors (Poland et al., 1999), in addition to differences in natural infections that may have occurred in these populations.

5. Genetically determined variability in vaccination responses

Two examples of genetically determined variability in vaccination responses have sprung to attention in the literature.

5.1 Measles

The relationship of the human leukocyte antigen (HLA) and transporter associated with antigen processing (TAP) genotype with antibody response to measles virus vaccination is shown in Table 1. Generally, non-responders (NR) show higher homozygosity rates. Regarding HLA class II, NR show a higher DR homozygosity rate and excess DR7, DRB1*07, and DQA1*05, whereas hyper-responders (HR) show excess DR13, DRB1*13, and DQA1*01.

Regarding HLA class I, an association of B7 and B51 with response was found, with a B7 allele dose response, whereas an inverse association was found with B13, B44, and C5.
NR are more likely to be homozygous at amino acid position 665 of TAP2.

5.2 Hepatitis B

The relationship of the HLA and complement genotype with antibody response to hepatitis B surface antigen (HBsAg) vaccination is shown in Table 2. NR showed increased homozygosity of the extended haplotype B8;SC01;DR3, where SC01 is a four-gene complement haplotype. Regarding HLA class II, NR show an increased frequency of Bw54;DR4;DRw53;DQw4, of DRB1*0701;DQA1*0201;DQB1*0201, of DPB*0201, and of DQB1*0604;DQA1*0102;DRB1*1302. In addition, an increased frequency of DRB1, DRB1*0405, *0406, *0802, *0401, and *1101, DQA1, DQB1, DPA1, DPA1*0103, DPB1, and DPB1*0402, 0202, and 1301 was seen for NR. HR showed an increased frequency of DQB1*0602;DQA1*0102;DR15 and of DQB1*0603;DQA1*0103;DRB1*1301. In addition, an increased frequency of DRB1*08032, *0101, and *1403, DQA1*0302, *0301, *0104, and *0601, DQB1*0401, *03032, and *0302, and DPB1*1401 was seen for HR.

Regarding HLA class I, NR showed an increased frequency of A, B, and A*2602;A*1101;B35;B70. HR showed an increased frequency of B46;B7.

Table 1. Summary Relationship between HLA or TAP and antibody response to measles virus vaccine.

Hyper-responder	**Non-responder**	
	higher DR homozygosity rate[1]	(Poland et al., 1995)
	higher homozygosity rate[2]	(Hayney et al., 1998)
	higher homozygosity rate[3]	(Poland et al., 1998)
excess DR13	excess DR7[4]	(Poland et al., 1995)
excess DRB1*13	excess DRB1*07[5]	(Hayney et al., 1996)
excess DQA1*01	excess DQA1*05[6]	(Hayney et al., 1998)
association with B7, B51	association with B13, B44, C5[7]	(Poland et al., 1998)
B7 allele dose response[8]		(Poland et al., 1998)
	more likely TAP665 homozygous[9]	(Hayney et al., 1997)

[1] 65 hyper-responders (HR)/81 nonresponders (NR); 39.5% vs. 13.8% (P = 0.0006)
[2] 64 HR/46 NR; 9.4% vs. 23.9% (P=0.037)
[3] 156 HR/140 NR; p=0.031; different HLA-B allele distribution (P = 0.002)
[4] 65 HR/81 NR; different DR allele frequencies (P = 0.014)
[5] N=881; 16.2% vs. 7.4% (p=0.02) and 6.2% vs. 15.4% (P = 0.015), respectively for HR vs. NR; different DRB1 allele distribution (P = 0.014)
[6] 64 HR/46 NR; P = 0.016 and P = 0.017, respectively; different allele frequency distribution (P = 0.05)
[7] 156 HR/140 NR; allele odds ratios 0.24 (P = 0.001), 0.19 (P = 0.018), ∝ (P = 0.009), 3.0 (P = 0.003), and 2.80 (P = 0.011), respectively
[8] 156 HR/140 NR; P < 0.0001
[9] 28 HR/32 NR; odds ratio 5.0 (P = 0.016)

Table 2. Relationship between HLA and antibody response to HBsAg vaccine.

Hyper-responder	**Non-responder/Hypo-responder**	
Caucasians		
	B8, SC01, DR3 homozygosity[1]	(Alper et al., 1989)
	DRB1*0701; DQA1*0201; DQB1*0201[2] DPB*0201[3]	(Martinetti et al., 1995)
	DRB1*0701;DQB1*0202[4]	(McDermott et al., 1997)
DRB1*01[5] DRB1*15[5]	DRB1*03[5] DRB1*14[5]	(Caillat-Zucman et al., 1998)
DRB1*010*[6] DR5[6] DPB1*040*[6] DQB1*0301[6] DQB1*0501[6]	DRB1*07[6] DPB1*1101[6] DQB1*020*[6]	(Desombere et al., 1998)
DRB1*13[7,8]	DRB1*3[7,8] DRB1*7[7]	(Höhler et al., 1998)
DQB1*0602; DQA1*0102;DR15[9] DQB1*0603;DQA1*0103;DRB1*1301[10]	DQB1*0604;DQA1*0102;DRB1*1302[11]	(Langö-Warensjö et al., 1998)
Japanese		
	Bw54-DR4-DRw53-DQw4[12]	(Watanabe et al., 1990)
	A, B, DRB1, DQA1, DQB1, DPA1, DPB1[13]	(Mineta et al., 1996)
A,B: B46; B7	A*2602; A*1101; B35; B70[14]	
DRB1: *08032, *0101, *1403	*0405, *0406, *0802, *0401, *1101[15]	
DQA1: *0302, *0301, *0104, *0601[16]		
DQB1: *0401, * 03032, *0302[17]		
DPA1:	DPA1*0103[18]	
DPB1: *1401	*0402, *0202, *1301[19]	

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[1] 15,608 U in heterozygotes (N = 9) vs. 467 U in homozygotes (N = 4) ($p < 0.01$)
[2] 23.5% (8/34) vs. 9.9% of controls (52/524)
[3] 42.3% (11/26) vs. 13.2% of controls (81/612)
[4] *86 NR, 131 HLA controls, and 117 vaccine controls: DRB1*07: NR vs. HLA controls: RR = 3.01 ($p < 0.001$); DQB1*02: NR vs. HLA controls: RR = 3.08 ($p < 0.001$), NR vs. vaccine controls: RR = 5.65 ($p < 0.001$). 30 NR upon revaccination: DRB1*07: NR vs. HLA controls: RR = 6.41 ($p < 0.001$); DQB1*02: NR vs. HLA controls: RR = 4.89 ($p < 0.001$).
*172 NR, 262 HLA controls, and 234 vaccine controls: DRB1*0701;DQB1*0202: NR vs. HLA controls: RR = 3.58 ($p < 0.001$), NR vs. vaccine controls: RR = 4.5 ($p < 0.001$). 60 NR upon revaccination. DRB1*0701;DQB1*0202: NR vs. HLA controls: RR = 5.8 ($p < 0.001$).
[5] 114 NR hemodialysed patients, 301 R hemodialysed patients, and 471 healthy controls, respectively: DRB1*01: 12.3% vs. 22.9% and 24.8% (OR = 0.47; p = 0.02); DRB1*15: 14% vs. 22.9% and 25.1% (OR = 0.55; p= 0.05); DRB1*03: 32.5% vs. 16.6% and 25.3% (OR = 2.42; p = 0.0007); DRB1*14: 9.6% vs. 3% and 6,6%

(OR = 3.46; p = 0.008). DRB1*03 vs. non-DRB1*03: p = 0.0007; DRB1*14 vs. non-DRB1*14: p = 0.02; DRB1*01 vs. non-DRB1*01: p = 0.018; DRB1*03 or DRB1*14 vs. DRB1*01 or DRB1*15: p < 0.0001.

[6]100 R and 46 NR. DRB1*010*: RR = 0.09 (p< 0.05). DR5: RR = 0.08 (p< 0.05). DRB1*0701-DRB4*0101: RR = 4.39 (p< 0.005). DPB1*040*: RR = 0.08 (p< 0.001). DPB1*1101: RR = 14.64 (p< 0.01). DQB1*020*: RR = 4.78 (p< 0.001). DQB1*0301: RR = 0.07 (p< 0.005). DQB1*0501: RR = 0.09 (p< 0.005).

[7]53 R and 73 NR: DRB1*3: p = 0.03. DRB1*7: p = 0.002. DRB1*13: p = 0.0001.

[8]56 R and 62 NR: DRB1*3: p = 0.0023. DRB1*13: p = 0.0375.

[9]69 R /53 NR; p < 0.025

[10]69 R/53 NR; p < 0.05

[11]69 R/53 NR; p < 0.001

[12]25 NR vs. 1998 control:

Bw54, p < 0.001, RR = 5.57
DR4, p < 0.05, RR = 2.81
DRw53, p < 0.001, RR = 21.24
Bw54-DR4, p < 0.001, RR = 6.35
DR4-DRw53, p<0.05, RR = 2.81

[13]N=339; R (multiple correlation coefficients of anti-HBs Ab production with each locus) =

[14]N=339; r (partial correlation coefficient of Ab production with each allele) = 0.11, 0.08, -0.14, -0.08, -0.09, and -0.20, respectively

[15]N=339; r = 0.17, 0.13, 0.08, -0.13, -0.10, -0.09, -0.12, and –0,10 respectively

[16]N=339; r = -0.19, -0.20, -0.08 and -0.08, respectively

[17]N=339; r = -0.18, -0.12, and -0.23, respectively

[18]N=339; r = 0.15

[19]N=339; r = -0.10, 0.16, 0.15 and 0.08, respectively

6. Age and vaccination responses

6.1 Vaccination response in childhood

Age is an important determinant for the immune response. In infants maturation of the immune system continues after birth. Neonates are not able to respond to most polysaccharide antigens; children after the age of two years do better. Also the response to protein antigens continues to further maturate during the first years of life (EPI, 1993). For this reason, infants receive four vaccinations as a basic immunization with DT-IPV (diphtheria, tetanus, and inactivated polio vaccine), while adults need only three for a similar effect. Circulating antibodies (from maternal origin, or from antibodies administered) impair the vaccination response, possibly by neutralization of vaccine antigens, or by a suppressor mechanism that down-regulates the antibody formation when sufficient antibodies are present. However, circulating antibodies appear not to prevent the antibody responses later in life (Halsey and Galazka, 1995; Sunderman et al., 1997). Interpretation of antibody levels as a parameter for the effect of external factors on immune responses needs consideration of this factor too.

6.2 Vaccination responses in the elderly

Using the SENIEUR-protocol (Ligthart et al. 1984), studies in well-characterized healthy elderly (> 65 yr.) populations (including history of illness, infections, drug intake, and laboratory values) have been performed, and show that the serum levels of IgG, IgM and IgA increase with age, as well as the number of benign monoclonal gammapathies and the number of auto-antibodies. The number of lymphocytes and their proliferative activity is decreased while the number of neutrophils increased with aging. Monocytes, basophils, and eosinophils are without changes during life but monocyte function was increased in elderly individuals (Rink et al., 1998; Rink and Seyfarth, 1997).

As a consequence of age-related alterations in the immune system, elderly may have an impaired response to primary as well as secondary immunization (Stein, 1994).

While the efficacy of influenza vaccine has been estimated to be 70-90% in young adults, it is lower in elderly nursing home patients (Gravenstein et al., 1992; Gross et al. 1995; Brandriss et al. 1981; Phair et al. 1978; Fagiolo et al. 1993). The diminished efficacy has been attributed to lower rates of protective antibody responses against the influenza strains. Haemagglutinin inhibition (HI) antibody titers of > 40 are generally considered protective. Yet, several studies indicate that at least 25% of the elderly do not develop HI-Ab titers after vaccination (Keren et al. 1988; Gross et al. 1988; Beyer et al. 1989).

Following vaccination elderly healthy subjects showed reduced production of anti-tetanus toxoid antibody, compared with young adults. Moreover the titers of elderly declined by 6 month to baseline values whereas in young adults titers persisted for up to one year (Burns et al. 1993). A recent study demonstrates that 40% of a population of SENIEUR-compatible Austrians were not protected against tetanus (Steger et al. 1996). Fifty percent of these individuals had been vaccinated within the last ten years, and 25% within the last five years.

Especially in the elderly, decreasing effectiveness (Shelly et al. 1997) with increasing delay since vaccination has been reported for pneumococcal vaccine (Sankilampi et al. 1997). The overall antibody response among elderly has been determined to be lower after re-vaccination than following primary vaccination (Mufson et al. 1991).

Vaccination procedures in the elderly generally comprise repeat vaccines, the vaccination response to these may be less adequate to use in studies of effects of environmental exposures on the immune system.

7. Effect of chronic psychological stress on the vaccination response

Negatively experienced chronic stress diminishes the efficacy of the immune system to protect the host against infections. Chronic stress leads to a decrease in natural killer (NK) cell number and activity, decreased lymphocyte response to mitogens, and an increase in CD4/CD8 ratio, and increases in virus infections and antibody titer to latent viruses.

In addition, the impairment of the immune response leads to a poorer response of the immune system to vaccines. The study of the response of individuals to vaccines is preferentially performed by using de novo antigens, as these are not affected by previous events. Various groups studied the effect of chronic psychological stress on the antibody (Ab) response to various vaccines and variable results were obtained (cf. TABLE 3). Generally, high levels of negative experienced stress (life events, academic exams, daily stress, and hassles) and anxiety appear to reduce the antibody response to a primary or secondary immunization with a vaccine.

Table 3. Effect of stress stimuli in vaccination studies.

Vaccine	Stressor	Observation	N	Reference
Hep. B	loneliness, hassles	lower Ab-response	95	Jabaaij, 1993
Hep. B	daily stress, neurotism	no effect on Ab-response	68	Jabaaij, 1996
Hep. B	life events, stress anxiety	higher peak AB-response	81	Petry, 1991
Hep. B	exam stress, social support	delayed Ab-response	48	Glaser, 1992
Influenza	alzheimer caregiving	lower Ab-response	64	Kiecolt-Glaser, 1996
Influenza	depression caregiving	lower IgG Ab-response	117	Vedhara, 1999
KLH	life events, daily sterss	lower IgG Ab-response	89	Snyder, 1990

The group of Jabaaij (Jabaaij et al. 1993) performed a study in stressed subjects, characterized by loneliness, daily hassles, psychoneurotic complaints, and coping style. Subjects were vaccinated and antibody titers determined seven months later. High stress score derived from the month of the second assessment was associated with a lower Ab response to the vaccine but in a later similar study, using a higher dose of the same vaccine, no effects were observed at any timepoint (Jabaaij et al. 1996).

Petry et al. (1991) vaccinated 81 seronegative subjects with a similar vaccine three times and determined antibody titers three months after the third dose i.e. in the booster phase of immunization. They showed that higher levels of negatively experienced stress, depression, irascibility, and anxiety during the 6-month period following the first vaccination were associated with higher peak antibody titers.

Kiecolt-Glaser et al. (1996) showed an impaired response in Alzheimer's caregivers (chronic stress) to influenza vaccination relative to matched controls. One month after vaccination 65% of the control subjects, and only 37% of the caregivers induced a four-fold increase in antibody response.

Similar results were recently observed in caregivers of dementia patients receiving a trivalent influenza vaccine (Vedhara et al. 1999). Mean scores of emotional distress were significantly higher in caregivers than in controls. In 26 of 67 controls (39%) and in only 8 of 50 care givers (16%) a four-fold increase in at least one of the IgG subclass titers was observed.

Glaser et al. (1992) studied the effects of stress on the antibody response to hepatitis B vaccine given three times to healthy students. The "early" seroconvertors were significantly less anxious and less stressed than "late" seroconvertors indicating that stress delayed the humoral immune response to hepatitis B vaccination.

Snyder et al. (1990) investigated the effect of stress and psychosocial factors on the antibody response to vaccination with KLH-antigen (keyhole limpet hemocyanin). Antibody titers were measured 3 and 8 weeks after immunization and showed that subject with more stressful events tended to have lower baseline and 3-week post-immunization IgG levels. Psychological distress scores correlated negatively and psychological well-being scores positively with IgG levels. Those who reported “good” tended to have higher IgG levels at 8 weeks post-immunization.

8. Nutrition and efficacy of vaccination

8.1 Malnutrition

It has been shown that immune responses can be affected in young children, depending on the severity of protein deficiency. Under extreme malnutrition conditions such as marasmic (severe caloric deficiency) and kwashiorkor (severe protein deficiency) impairment of vaccination was found for yellow fever, smallpox (Anonymous, 1967), tuberculosis, and polio (Sinha et al., 1976; Adeiga et al., 1994). No impairment of the immune response to the vaccination was found under mild and moderate conditions of malnutrition on vaccination against tuberculosis, measles (Ifekunigwe et al., 1980; Monjour et al., 1984) smallpox (Anonymous, 1967; Ifekunigwe et al., 1980), yellow fever (Anonymous, 1967), diphtheria, tetanus, and pertussis (Kielmann et al., 1976).

Malnutrition caused by anorexia nervosa or bulimia nervosa was associated with disturbances in the immune system (Marcos et al., 1997a, b). A general decrease in lymphocyte subsets, except for CD19+ cells (B-cells) is described for anorexia and bulimia nervosa patients. In addition impairment for the cell-mediated response (DTH) was found in anorexia nervosa patients (Marcos et al., 1997a). It is noteworthy that anorexia nervosa patients are not prone to infections (Golla et al., 1981; Cason et al., 1986; Schattner et al., 1999).

8.2 Breast feeding versus formula feeding

Breast and artificial milk are the major nutrition during the first year of life. The effect of breast milk and four types of artificial feed on the effect of vaccination was studied. It was found that babies fed on breast milk or high -protein cow milk had an adequate and sustained responses; those fed on formula that were relatively low on proteins and carbohydrates had high but temporary responses and those fed on low protein cow milk or the soy-based formula had poor responses (Lesourd, 1995). Besides protein content of the type of feeding, contaminants in the feeding may also have had an influence.

8.3 Food constituents

It is known that the presence or the absence of certain constituents in nutrition can affect immune responses (Panush and Delafuente, 1985; Middleton and Kandaswami, 1992; Bendich, 1996; Kelly and bendich, 1996). Addition of vitamins C and E to food has been shown to stimulate immune responses, while suppressed immune responses have been observed associated with deficiency of vitamins A, B, and E. Alterations of immune responses are also found by iron and zinc deficiency. These trace metals are essential for the development and maintenance of the cell mediated (iron and zinc) and humoral response (iron). In general, it appears that cell-mediated and nonspecific immunity are more sensitive for nutrition deficiency than humoral immunity (Scrimshaw and sanGiovanni, 1997).

Today, there is a growing interest in diets specifically designed to promote health of the consumer. Probiotics such as lactic acid bacteria can transiently colonize the intestine and

exert beneficial effects on the immune system (Schiffrin et al., 1997). Fish oil, which is rich in eicosapentanoic and docosahexaenoic acid, affects cell-mediated and the humoral responses in both humans and experimental animals, some stimulated and others down regulated (Virella et al., 1991; Calder, 1997).

In elderly the effect of immunosenescence is superimposed on the development of malnutrition. Randomized controlled studies have shown that supplementation of vitamin E for 4 months improved certain clinically relevant indexes of the cell-mediated immunity in healthy elderly. Delayed-type hypersensitive and antibody titers to hepatitis B were significantly increased, as were antibody titers to tetanus vaccination (Lesourd, 1995; 1997; Meydani et al., 1997). In a study of influenza vaccination in the elderly with low serum albumin levels a very poor antibody response to the influenza vaccination was induced, (Lesourd, 1995) while in another study no difference was observed between elderly and young adults (Pozzetto et al., 1993).

In conclusion, the data indicate that nutritional status as well as individual nutritients in the food can affect vaccination titers, and should therefore be a concern in the design of epidemiological studies of effects of environmental factors on the immune system.

9. The influence of infectious diseases on the immune response to vaccination

Numerous inflammatory and immune reactions that occur in response to infection, might -in theory- affect the outcome of a vaccination given in the course of that infection. Pathogens may affect the immune response following vaccination by infecting CD4+ Th cells and macrophages. This has been documented for viruses (human immunodeficiency virus (HIV), measles virus, enteroviruses), bacteria (*Streptococci* and *Staphylococci*), and parasites (*Leishmania, Plasmodium*). They may further influence the immune system by stimulating the production of cytokines, which in turn may affect the nature and magnitude of the immune response following vaccination (Brenan and Zinkernagel 1983, Kotwal et al 1997).

9.1 Influence of immunosuppressive infections on the vaccination response

HIV, measles virus, some bacteria (*Salmonella*) and helminthes (*Schistosoma, Nematospiroides*) exert well-documented immunosuppressive effects. In addition, it is well known that HIV-infected persons may have a poor response upon vaccination against measles virus, hepatitis A and B virus (Bouchaud and Mouas 1998, Tilzey et al, 1996, Loke et al 1990, Arpadi et al 1996).

Infection with *Plasmodium* spp. has several effects on the function of immune cells and has been documented to inhibit the antibody response to tetanus toxoid (Dietz 1997).

Measles virus is clearly immunosuppressive. It interferes with the function of antigen-presenting cell as monocytes and dendritic cells. This may lead to deficiencies in IL-12 production and T cell proliferation (Karp et al 1996, Bell et al 1997, Fugier-Vivier et al 1997). Despite these well-documented immunosuppressive effects of measles virus, the effect of measles virus infection on concurrent vaccination is not documented.

Chronic carriers of hepatitis B virus have a disturbed T helper cell function, which is associated with a reduced recall response to whole tetanus toxoid (Livingston et al 1999). The effect of chronic hepatitis B virus carriership on primary vaccinations is unknown.

The same is true for helminth and bacterial infections. *Salmonella*, *Schistosoma,* and *Nematospiroides* may influence the function of B and T cells (Actor et al 1993, al-Ramadi et al 1992, Pritchard et al 1984). Yet their influence on the vaccination response is not documented.

9.2 Influence of non-immunosuppressive and non-specified infections on the vaccination response

Oral poliovirus vaccine (OPV), a live attenuated poliovirus, interferes with the antibody response to a rotavirus vaccine. However, the effect was small and could be circumvented by a higher dose of vaccine (Rennels 1996).

A number of studies have been directed on the question whether non-specified infections, manifested by symptoms such as diarrhea, rhinorrhoea, coughing, fever, rash, or a febrile upper respiratory tract infection, negatively affect the vaccination response against mumps, measles, rubella, and poliovirus. One study described a negative effect (Migasena et al 1998), while seven other studies reported no or only minimal, clinically insignificant influence (Faden and Duffy 1992, Scott et al 1999, Halsey et al 1985, Cilla et al 1996, Edmonson et al 1996, Ratnam et al, 1995, Dennehy et al 1994).

9.3 Specific interaction with cross-reacting pathogens

Infection with microorganisms that are closely related to vaccine components may interfere with the vaccination response to such components, for example because they cross-react or limit the replication of vaccine virus. Sabin OPV type 2, for example, interferes with the vaccination response to Sabin type 3 (Maldona et al 1997). Non-polioenteroviruses may also interfere with the vaccination response to OPV. In contrast, non-specific enteric infection did not interfere with OPV vaccination (Triki et al 1997).

In conclusion, although some infections may exert well-documented immunosuppressive effects in either humans or laboratory animals, their influence on the vaccination response appears only poorly documented. The influence of well-known immunosuppressive infections, such as measles virus and HIV, appears limited in well-developed countries such as the Netherlands, because their incidence is very low. Clinical measles virus infection in the Netherlands is limited to persons who refuse vaccination on religious grounds. The influence of non-specified childhood infections on the vaccination response has been evaluated in several studies. These infections appear to have no or only a limited negative influence on the response to vaccination.

10. Discussion and concluding remarks

The literature to date indicates that many influences on the response to a vaccination exist, and that these therefore should be taken into consideration in the design of epidemiological studies aimed at assessing the effect of environmental exposures on which the response to vaccination is employed as a read out of the function of the immune system. In other words, these influences need to be carefully controlled for.

It is necessary to know the inter- and intra-person variability in the population regarding the responses to the chosen vaccine, so that the required study group size can be determined.

It appears that the vaccine itself, the vaccination route, booster vaccination, and time point during or after completion of the vaccination procedure have impacts on the vaccination titers that will be observed. Therefore, if vaccination titers are used as a read out in epidemiological studies, it is of importance to standardize these. Moreover, insight in the interval between the commencement of the exposure that is being studied, and the effect on the immune system that is expected (hours, days, months, years) will help to design the study.

The data indicate that genetics, age, psychological stress, smoking, nutrition, and certain infectious diseases that are not necessarily directly antigenically related to the vaccine all may have an influence on vaccination titers, and should be considered as confounders. Also, geographic differences have been noted, which may have several causes, among others of socio-economic or cultural nature. Little information is available on the quantitative relevance of all these confounders, and therefore studies need to be designed so that either these confounders are excluded, or that it is possible to correct for these influences. It is obvious that such influences have differential relevance in different populations, such as e.g. elderly vs. children, or populations in wealthy societies vs. underdeveloped regions.

According the extensive animal studies reported by Luster et al (1992, 1993) the antigen-specific response to T cell dependent antigens (sheep erythrocytes) correlates well with host resistance to infectious diseases. It is quite likely that this applies for humans as well. In those cases where reduced antibody titers to a given vaccination are observed, that cannot be attributed to any other determinant than the environmental immunosuppressive agent that one studies, it is likely that resistance to infections in the population due to this exposure is negatively influenced. Obviously, there is a certain reserve capacity, and not every change in function of the immune system will lead to a decreased resistance in healthy individuals. Yet, since in the entire population a high prevalence of different types of infectious diseases, such as common colds, gastroenteritis, etc is evident, further suppression of immune responses in infected individuals is likely to have an impact. These impacts may be expressed as prolonged duration or more severe disease due to the infection. Such effects may go unnoticed, since they may only lead to more of the same symptoms. They may actually not be very significant for the individual patient. But due to the high prevalence of many rather innocent infections, such effects may on a population basis be very significant.

With respect to the efficacy of vaccination in terms of protection, it needs to be mentioned that it is not always possible to deduct from the vaccination titer the level of protection that is gained. It is therefore likewise not possible to deduct from effects of environmental factors as

they may be encountered, whether these effects hamper protection against the pathogen at which the vaccination is aimed.

Antibody response after hepatitis B immunization, however, predicts susceptibility to disease on exposure (Hadler et al., 1986, McMahon and Wainwright, 1993). This is also true for post-immunization measles antibody responses and for post-immunization polio antibody responses. Responses in the “low positive” range do not protect against clinical measles when subjects are exposed to the wild measles virus, whereas high levels are protective (Chen et al., 1990). A strong correlation exists between low antibody levels after a single dose of (measles) vaccine and high susceptibility to infection with exposure (Deseda-Tous et al, 1978). So any insult to responses to these vaccines that result in titers beneath a certain threshold will be indicative of effects even for the protection at which the vaccine is aimed. It is, however, unlikely that such conditions will readily be encountered. For the majority of the population, vaccination is performed so, that modest or even relatively big variations in the response do not result in altered protection, albeit that not every individual will always be protected. This is corroborated by the findings in developing countries in which malnutrition of children shows impaired responses after vaccination, whereas these alterations do not cause the general failure of vaccination strategies (even though it should be mentioned that sometimes problems with vaccination to measles are encountered that are associated to vitamin A deficiency). One may expect, that individuals with a response to a vaccine that leads to borderline protection, may be subject to experiencing a clinically significant negative consequence of diminished vaccination response if environmental exposure that affects vaccination responses occurs.

In conclusion it can be stated that vaccination titers may be applied to study effects of exposures to environmental factors, provided that confounders are adequately controlled for. Variability in the response to vaccination are likely to be smallest in the case of vaccination to an antigen to which no prior exposure, either naturally or by prior vaccination has occurred, which may apply especially to vaccination in children. In addition, confounders such as stress or smoking may also be less evident in children. For this reason, vaccination in children may prove to be most adequate to study immune effects of environmental factors.

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Appendix 1 Mailing list

1. Directeur-Generaal RIVM
2. Dr. H. Roelfzema, GZB/CO
3. Dr. P.C. Bragt, ST.TZ/W&V
4. Dr. J.A.M. Whyte, GZB/CO
5. Dr. H. de Sitter, ST.TZ/W&V
6. Dr. C. Colosio, International Centre for Pesticide Safety, Italy
7. Prof. F.P. Kaloyanova - Simeonova, National Center of Hygiene, Medical Ecolgy and Nutrition, Bulgaria
8. Prof. C. la Vecchia, Instituto di Richerche Farmacologiche “Mario Negri”, Italy
9. Prof. J. Liesivuori, University of Kuopio, Finland
10. Prof. D. Trichopulos, University of Athens, Athens
11. Dr. J.F. Acquavella, Monsanto Company, U.S.A.
12. Dr. M.M. Amr, Cairo University, Egypt
13. Dr. P. Ayotte, Public Health Center Québec, Canada
14. Dr. S. S. Baliga, National Academy of Sciences, U.S.A.
15. Dr. I.L. Bernstein, University of Cincinnati Medical Center, U.S.A.
16. Dr. R. Biagini, Robert A. Taft Laboratories, U.S.A.
17. Dr. R. Crevel, Unilever Research Laboratory, U.K.
18. Dr. J.H. Dean, Sanofi Research, U.S.A.
19. Prof.Dr. J. Descotes, Hôpital Edouard Herriot, France
20. Dr. J.L. Devalia, The London Chest Hospital, U.K.
21. Dr. M. Dostal, Laboratory of Genetic Ecotoxicology, Czech Republic
22. Dr. A. Emmendörffer, Fraunhofer Institut für Toxicologie und Aerosolforschung, Germany
23. Dr. G. Eijkemans, World Health Organisation, Switzerland
24. Dr. D.R. Germolec, National Institute of Environmental Health Sciences, U.S.A.
25. Dr. R. Glaser, The Ohio StateUniversity Medical Center, U.S.A.
26. Dr. A.J. Hall, University of London, U.K.
27. Prof. Dr. med. R. Hess, University of Basel, Switzerland
28. Dr. M. Holsapple, The Dow Chemical Company, U.S.A.
29. Dr. R. Hong, The University of Vermont, U.S.A.
30. Dr. R. Delon Hull, NIOSH, U.S.A.
31. Dr. L. Kovtiuh, Toronto, Canada
32. Dr. S.E. Loveless, Dupont Co., Haskell Laboratory, U.S.A.
33. Dr. M. Løvik, National Institute of Public Health, Norway
34. Dr. M.I. Luster, Centers for Disease Control and Prevention, U.S.A.
35. Dr. J.S. Mandel, University of Minnesota, U.S.A.
36. Prof.Dr. A.J. McMichael, London school of Hygiene and Tropical medicine, U.K.

37. Dr. T. Meredith, World Health Organisation, Switzerland
38. Dr. T. Nicolai, Dr. Von Haunersches Kinderspital, Germany
39. Dr. C. Nolan, European Commission, Brussels
40. Dr. H. Pastides, University of Massachusetts Amherst, U.S.A.
41. Dr. M.K. Selgrade, National Health and Environmental Effects Research Laboratory, U.S.A.
42. Dr. P.K. Seth, Industrial Toxiclogy Research Centre, India
43. Dr. R.F. Vogt, Centers for Disease Control, U.S.A.
44. Dr. B. Xue, Beijing Medical University, P.R. China
45. Dr. M. Younes, World Health Organisation, Switzerland
46. Prof.Dr. H. Brunekreef, Agricultural University, the Netherlands
47. Dr. H. Houweling, Gezondheidsraad, the Netherlands
48. Prof.Dr. R. Kroes, RITOX, Universiteit Utrecht, the Netherlands
49. Dr. R.H.H. Pieters, RITOX, Universiteit Utrecht, the Netherlands
50. Prof.Dr. B. Zegers, UMC-Wilhelmina Childrens Hospital, the Netherlands
51. Dr. N. Weisglas - Kuperus, Sophia Childrens Hospital, the Netherlands
52. Depot Nederlandse Publikaties en Nederlandse Bibliografie
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55. Dr.Ir. G. de Mik, directeur sector ¾
56. Dr. A.D. Plantinga, hoofd LVO
57. Dr. H. van Loveren, projectleider/auteur
58. Dr. J.G.C. van Amsterdam, auteur
59. Dr. R.J. Vandebriel, auteur
60. Dr. T.G. Kimman, hoofd LIO/auteur
61. Dr. H.C. Rümke, auteur
62. Dr. P.S. Steerenberg (LPI), auteur
63. Prof.Dr. J.G. Vos, hoofd LPI/auteur
64. Dr. Ir. E. Lebret (LBM)
65. Drs. F. Termorshuizen (LPI)
66. Dr. W.H. de Jong (LPI)
67. Dr. J. Garssen, LPI
68. SBD/Voorlichting en Public Relations
69. Bureau Rapportenregistratie
70. Bibliotheek RIVM
71-85. Bureau Rapportenbeheer