Vaccine-Induced Antibody Responses as Parameters of the Influence of Endogenous and Environmental Factors

Henk Van Loveren, Jan G.C. Van Amsterdam, Rob J. Vandebriel, Tjeerd G. Kimman, Hans C. Rümke, Peter S. Steerenberg, and Jeff G. Vos

National Institute of Public Health and the Environment, Bilthoven, the Netherlands

In laboratory animals, an adequate way to assess effects of environmental exposures on the immune system is to study effects on antigen-specific immune responses, such as after sensitization to T-cell-dependent antigens. This probably also applies to testing effects in the human population. It has thus been suggested that antibody responses to vaccination might be useful in this context. Vaccination responses may be influenced by a variety of factors other than environmental ones. One factor is the vaccine itself; a second is the vaccination procedure used. In addition, the intrinsic capacity of the recipient to respond to a vaccine, which is determined by sex, genetic factors, and age, is important. Psychological stress, nutrition, and (infectious) diseases are also likely to have an impact. We reviewed the literature on vaccine response. With regard to exogenous factors, there is good evidence that smoking, diet, psychological stress, and certain infectious diseases affect vaccination titers, although it is difficult to determine to what extent. Genetic factors render certain individuals nonresponsive to vaccination. In general, in epidemiologic 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 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 performed. Only in those cases where individuals have a poor response to the vaccine may exogenous factors perhaps have a clinically significant influence on resistance to the specific pathogen. An exposure-associated inhibition of a vaccination response may, however, signify a decreased host resistance to pathogens against which no vaccination had been performed. Key words: age, antibody responses, epidemiology, genetic factors, immunotoxicity, nutritional factors, stress, vaccination. Environ Health Perspect 109:757-764 (2001). [Online 31 July 2001] http://ehpnet1.niehs.nih.gov/docs/2001/109p757-764vanloveren/abstract.html

Antibody responses to vaccination are influenced by a variety of endogenous factors including genetics, sex, age, and exogenous factors such as stress, nutrition, and infectious diseases. These factors need to be taken into consideration in clinical and epidemiologic studies where the antibody response is the biomarker 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 the International Programme on Chemical Safety (1)]. Effects of environmental exposures on immune functions have also been shown in humans (1), 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 subtle immunosuppression, consequences of such suppression may become evident as increased incidences of common infections, such as influenza and common cold (1).

In experimental studies in rodents, it has been shown that the antibody response to sheep erythrocytes are a valuable indicator for immunotoxicity (2,3). 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, alterations in the response to sheep erythrocytes correlates well with resistance to experimental infectious agents in these animal studies (3). This immune function test was also applied in nonhuman primates; for example, exposure of female rhesus monkeys (Macaca mulatta) to polychlorinated biphenyls (PCBs) reduced the antibody response to sheep erythrocytes in conjunction with effects on several other immunologic parameters (4).

Of course, it is not possible to use antibody titers to sheep erythrocytes to study immunotoxicity in human populations. It has therefore been suggested that effects of immunotoxic exposures on the specific immune response to agents derived from infectious microorganisms, (e.g., to vaccines) be used instead (5,6). Depending on the vaccine, major components of the immune system may be involved in the ultimate humoral response to the vaccine. It is not possible for all vaccination responses to translate the vigor of the antibody response to protection. Ideally, vaccination is performed in such a way that those variations in response do not result in altered protection, but 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.

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 (7,8).

Also, such consequences of environmental exposures have been documented in the literature. For instance, children born between July 1978 and June 1987 to mothers that had been exposed to toxic levels of PCBs and dibenzofurans through consumption of contaminated rice bran oil in 1978–1979 showed higher frequencies of upper respiratory tract infections (5).

Address correspondence to H. Van Loveren, National Institute of Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, the Netherlands. Telephone: +31-30-2742476. Fax: +31-30-2744437. E-mail: h.van.loveren@rivm.nl

We thank E. Lebret, J. Smit, F. Termorshuizen, A. Wijga, and B. van der Zeejts for critically reviewing the manuscript.

Received 28 December 2000; accepted 1 February 2001.
Exposure to organochlorines through the food chain in Inuit mothers in Nunavik (Arctic Quebec) has been reported to increase the incidence of otitis media in their breast-fed children (9). An association of cumulative background exposure to PCBs and dioxins on the prevalence of otitis media was also reported in a group of 3.5 year olds in the Netherlands (10). In addition, an association of the prevalence of chickenpox with maternal exposure was observed.

Few studies have used vaccination titers to detect immunotoxicity in humans. This holds true even for studies of immunosuppression by pharmaceuticals such as cyclosporin. 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 (11). After treatment for acute leukemia, children had reduced antibody responses to diphtheria, tetanus, and inactivated polio vaccine (DT-IPV) (12). Also post-transplant (organ, bone marrow) immune suppression has been shown to lead to a long period of hyporesponsiveness to vaccinations (13–15).

A vaccination response study that did not involve humans was performed in seals by De Swart et al. (16). 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 PCBs, was 10-fold higher in Baltic compared to Atlantic herring. In seals that were fed with the Baltic herring, a significantly decreased specific antibody response to ovalbumin was observed.

In a study by Rieger and Graber (17), 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, although lead certainly has been identified as an immunotoxicant (18).

One example of the role of lifestyle factors in antibody responses in humans is the effect of smoking. Increased specific serum IgA and IgG responses to Chlamydia pneumoniae were observed by Von Hertzen et al. (19,20). These responses were to the natural infection by the pathogen and not to a vaccine. 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 suboptimal responses to vaccination with hepatitis B (21,22). In contrast, elevated antibody titers to influenza vaccination were noted in smokers (23).

A final example of studies on effects of environmental exposures on specific antibody titers after vaccination is the studies performed in the Netherlands by Wiegel and colleagues (10,24). They observed lower antibody responses to measles and rubella in some breast-fed infants. At 3.5 years of age, there was a statistically significant negative correlation of antibody titers to measles vaccination with the exposure to PCBs and dioxins as determined in cord blood, and a statistically significant negative correlation of antibody titers to rubella with maternal exposure to these compounds. However, after correction for sex, early feeding type (formula fed or breast-fed), duration of breastfeeding 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.

In a study by Termorshuizen et al. (25), 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 the winter 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: exposure to ultraviolet radiation diminished antibody titers after vaccination, and ultraviolet radiation exposure was 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.

Variability in Vaccination Titers due to the Vaccination

A number of factors related to the way vaccination is performed determine the qualitative and quantitative immune response to the vaccine. The first is number of vaccine doses (in the case of nonreplicating vaccines). In 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 (26), and thereafter confirmed in numerous studies (27).

The second factor is spacing of doses. In infant vaccination schedules that have longer intervals between doses, the postvaccination antibody titers are usually higher than in short-spaced vaccination schedules. Short-spaced vaccination series however induce protection earlier (26–28). A third factor is 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 (27,29). In practice, this is only relevant for vaccines that are available in a range of concentrations (depending on the indications), such as hepatitis A and hepatitis B vaccines.

Finally, kinetics of the immune response are important. A first dose of an inactivated vaccine often does not induce a detectable antibody response, yet it does prime B- and T-memory cells. There is a vigorous response 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 affect the vaccination titers. Therefore, if vaccination titers are used as an indicator in epidemiologic studies, it is important to account for these variables.

Sociogeographic Effects

In some vaccine studies using the same lots of vaccines and schedules, the response in one group is higher than in another group (e.g., in Turkish vs. Belgian infants, in Apeldoorn vs. Rotterdam infants), suggesting that genetic and/or environmental factors affect circulating antibody levels after vaccination (30).

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 (31), in addition to differences in natural infections that may have occurred in these populations.

Genetically Determined Variability in Vaccination Responses

Two examples of genetically determined variability in vaccination responses have been reported in the literature (32–45).

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, nonresponders had higher rates of homozygosity. Regarding HLA class II, nonresponders had a higher homozygosity rate
Vaccination responses and environmental factors

Environmental Health Perspectives • VOLUME 109 | NUMBER 8 | August 2001

759

of the DR locus, and excess of DR7 alleles, a DRA1 allele, and a DQA1 allele. Hyper-
responders had excess of DR13 alleles, (another) DRB1 allele, and (another) DQA1 allele.

Regarding HLA class I, an association of two B alleles with response was found, with
an allele dose response in one case, whereas an inverse association was found with two
(other) B alleles and a C allele. Non-
responders were more likely homozygous at a specific amino acid position of TAP2.

Hepatitis B. The relationship of the HLA and complement genotype with anti-
body response to hepatitis B surface antigen vaccination is shown in Table 2. Regarding
HLA class II, nonresponders showed an increased homozygosity for a combination
of two alleles. An increased frequency of a wide range of (single) alleles was found, and also
combinations of three or four alleles were found. For high responders, increased fre-
cuencies of a wide range of (single) alleles was also found, as well as combinations of
three alleles. Regarding HLA class I, in non-
responders as well as in high responders, increased frequencies of several (single) alleles
were found. Nonresponders showed increased homozygosity for a specific combi-
nation of three haplotypes, one for class I, one for class II, and one for complement.

In conclusion, several HLA class I and
class II genes are involved in the response to
vaccination against measles and hepatitis B.

For measles, a polymorphism in TAP may
also be involved.

Age and Vaccination Responses

Vaccination response in childhood. Age is an
important determinant for the immune
response. In infants, maturation of the immune system continues after birth.

Nononates are not able to respond to most
polysaccharide antigens; children do better after 2 years of age. Also, the response to
protein antigens continues to further matu-
rate during the first years of life (28). For
this reason, infants receive four vaccinations
as a basic immunization with DT-IPV, while adults need only three for a similar effect.

Circulating antibodies (from maternal origin or from antibodies administered) impair the
vaccination response, possibly by neutraliz-
ing vaccine antigens or by a suppressor
mechanism that downregulates the antibody
formation when sufficient antibodies are present. However, circulating antibodies appear
to not prevent the antibody responses later in life (46). Interpretation of antibody levels
as a parameter for the effect of external fac-
tors on immune responses needs considera-
tion of this factor, too.

Vaccination responses in the elderly. Using the SENIEUR-protocol (47), studies in
well-characterized, healthy elderly (> 65 years) populations (including history of ill-
ness, infections, drug intake, and laboratory values) have been performed and showed
that the humoral levels of IgG, IgM, and IgA increase with age, as well as the number of
benign monoclonal gammapathies and the number of autoantibodies. The number of
lymphocytes and their proliferative activity decreases, while the number of neutrophils
increases with aging. Monocytes, basophils,
and eosinophils do not change during life,
but monocyte function was increased in
elderly individuals (48,49).

As a consequence of age-related alter-
tations in the immune system, the elderly may
have an impaired response to primary as well as secondary immunization (50). The
efficacy of influenza vaccine has been estimated to be
70–90% in young adults, but it is lower in
elderly nursing home patients (51-55). The
diminished efficacy has been attributed to
lower rates of protective antibody responses
against the influenza strains. H emagglutinin
inhibition antibody titers of > 40 are gener-
al considered protective. Yet, several studies
indicate that at least 25% of the elderly do
do not develop hemagglutinin inhibition anti-
body titers after vaccination (52,56,57).

Following vaccination, elderly, healthy
subjects showed reduced production of anti-
tetanus toxoid antibody, compared with young adults. Moreover, the antibody
titers of the elderly declined by 6 months to baseline
values, whereas in young adults titers
persisted for up to 1 year (58). A recent
study demonstrates that 40% of a popula-
tion of SENIEUR-compatible Austrians
were not protected against tetanus (59). Fifty
percent of these individuals had been vacci-
nated within the last 10 years and 25%
within the last 5 years.

Especially in the elderly, decreasing effec-
tiveness (60) with increasing delay since vac-
cination has been reported for pneumococcal
vaccine (61). The overall antibody response
among the elderly has been determined to be
lower after revaccination than after primary

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

| Hyperresponder | Nonresponder | Reference |
|----------------|--------------|-----------|
| Excess DR13    | Excess DR7   | (32)      |
| Excess DRB1*13 | Excess DRB1*07 | (35) |
| Excess DQA1*01 | Excess DQA1*05 | (33) |
| Association with B7, B51 | Association with B13, B44, C5 | (34) |
| B7 allele dose response | More likely TAP665 homozygous | (36) |

Table 2. Relationship between HLA and antibody response to HBsAg (hepatitis B surface antigen) vaccine.

| Hyperresponder | Nonresponder/hyporesponder | Reference |
|----------------|----------------------------|-----------|
| Caucasian      | B8, SC01, DR3 homozygosity | (37)      |
| DRB1*0701; DQA1*0201; DQB1*0201 | DRB1*0201 | (38) |
| DRB1*0202 homozygosity | DRB1*03 | (39) |
| DRB1*0701; DQA1*0201; DQB1*0201 | DRB1*14 | (40) |
| DPB1*1101 | DPB1*0201 | (41) |
| DPB1*0400 | B7 allele dose response | (42) |
| DRB1*0501 | DQB1*0604; DQA1*0102; DRB1*1302 | (43) |
| DRB1*13 | DRB1*0603; DQA1*0103; DRB1*1301 | (44) |
| Japanese      | Bw54,DR4,DRw53,Do24 | (45) |
| A,B, DRB1, DQA1, DQB1, DPA1, DPB1 | A*2602, A*1101, B35, B70 | (46) |
| *08032, *0101, *1403 | *0405, *0406, *0802, *0401, *1101 | (47) |
| *0302, *0301, *0104, *0601 | *0401, *0302, *0302 | (48) |
vaccination (62). Vaccination procedures in the elderly generally consist of repeat vaccines, and the response to these vaccinations may be less adequate to use in studies of effects of environmental exposures on the immune system.

Effect of Chronic Psychological Stress on the Vaccination Response

Objective scores of chronic stress such as loneliness, psychoneurotic complaints, depression, irascibility, and anxiety have been used to study effects of stress on the immune system in humans (63–70). Chronic stress diminishes the efficacy of the immune system to protect the host against infections. Chronic stress leads to a decrease in natural killer cell number and activity, decreased lymphocyte response to mitogens, 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. Varying groups studied the effect of chronic psychological stress on the antibody response to various vaccines, and variable results were obtained (Table 3). Generally, high levels of stress (negative life events, academic exams, daily stress) and anxiety appear to reduce the antibody response to a primary or secondary immunization with a vaccine.

Jabaji et al. (63) performed a study in stressed subjects characterized by loneliness, daily hassles, psychoneurotic complaints, and submissive coping style. Subjects were vaccinated and antibody titers determined 7 months later. A high stress score derived in the month of the second assessment was associated with lower antibody response to the vaccine, but in a later, similar study using a higher dose of the same vaccine, no effects were observed at any time point (64).

Petry et al. (65) vaccinated 81 seronegative subjects with a similar vaccine three times and determined antibody titers 3 months after the third dose (i.e., in the booster phase of immunization). Higher levels of stress, depression, irascibility, and anxiety during the 6-month period following the first vaccination were associated with higher peak antibody titers.

Glaser et al. (66) studied the effects of stress on the antibody response to hepatitis B vaccine given three times to healthy students. The “early” seroconverters, being significantly less anxious and less stressed than “late” seroconverters, indicating that stress delayed the humoral immune response to hepatitis B vaccination.

Kiecolt-Glaser et al. (67) found impaired responses in Alzheimer’s caregivers (subject to chronic stress) to influenza vaccination relative to matched controls. One month after vaccination, 65% of the control subjects, but only 37% of the caregivers, had a 4-fold increase in antibody response.

Similar results were recently observed in caregivers of dementia patients receiving a trivalent influenza vaccine (68). Mean scores of emotional distress were significantly higher in caregivers than in controls. In 26 of 67 controls (39%), but in only 8 of 50 caregivers (16%), a 4-fold increase in at least one of the IgG subclass titers was observed.

Snyder et al. (69) investigated the effect of stress and psychosocial factors on the antibody response to vaccination with KLH (keyhole limpet hemocyanin) antigen. Antibody titers were measured 3 and 8 weeks after immunization, and anxiety and stress levels of subjects with more stressful events tended to have lower baseline and 3-week postimmunization IgG levels. Psychological distress scores correlated negatively and psychosocial well-being scores correlated positively with IgG levels. Those who reported less stress tended to have higher IgG levels at 8 weeks postimmunization.

Nutrition and Efficacy of Vaccination

Malnutrition. Protein deficiency can affect immune responses in young children, depending on its severity. Under extreme malnutrition conditions such as marasmus (severe caloric deficiency), and kwashiorkor (severe protein deficiency) impairment of vaccination was found for yellow fever, smallpox (70), tuberculosis, and polio (71, 72). No impairment of the immune response to the vaccination was found under mild and moderate conditions of malnutrition on vaccination against tuberculosis, measles (73, 74) smallpox (70, 73), yellow fever (70), diphtheria, tetanus, and pertussis (75).

Malnutrition caused by anorexia nervosa or bulimia nervosa was associated with disturbances in the immune system (76, 77). A general decrease in lymphocyte subsets, except for CD 19+ cells (B cells) is described for anorexia and bulimia patients. In addition, impairment for the cell-mediated response (delayed-type hypersensitivity) was found in anorexia patients (76). It is noteworthy that anorexia nervosa patients are not prone to infections (78–80).

Breast-feeding versus formula feeding. Breast and artificial milk are the major nutrition during the first 6 months of life, and still are important in later months. Lesourd (81) studied the effect of breast milk and four types of artificial milk on the effect of vaccination. Babies fed breast milk or high-protein cow’s milk had an adequate and sustained responses; those fed on formula that was relatively low on proteins and carbohydrates had high but temporary responses, and those fed on low-protein cow’s milk or the soy-based formula had poor responses (81). Besides protein content, contaminants in the formula may also have had an influence.

Food constituents. The presence or the absence of certain vitamins and nutrients can affect immune responses (82–85). Addition of vitamins C and E to food has been shown to stimulate immune responses, and suppressed immune responses have been observed associated with deficiency of vitamins A, B, and E. Immune responses are also affected by iron and zinc deficiencies. 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 to nutrition deficiency than humoral immunity (86).

Currently, there is a growing interest in diets specifically designed to promote health. Probiotics such as lactic acid bacteria can transiently colonize the intestine and exert beneficial effects on the immune system (87). Fish oil, which is rich in eicosapentanoic and docosahexanoic acid, affects cell-mediated and humoral responses in both humans and experimental animals, with some stimulated and others down-regulated (88, 89).

In the 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 indices of cell-mediated immunity in healthy elderly persons. Delayed-type hypersensitivity and antibody titers to hepatitis B were significantly increased, as were antibody

| Vaccine     | Stressor                     | Observation          | No.  | Reference |
|-------------|------------------------------|----------------------|------|-----------|
| Hepatitis B | Loneliness, hassles          | Lower Ab-response    | 95   | (63)      |
| Hepatitis B | Daily stress, neuroticism    | No effect on Ab-response | 68   | (64)      |
| Hepatitis B | Life events, stress, anxiety | Higher peak Ab-response | 81   | (65)      |
| Hepatitis B | Exam stress, social support  | Delayed Ab-response  | 48   | (66)      |
| Influenza   | Alzheimer caregiving         | Lower Ab-response    | 64   | (67)      |
| Influenza   | Depression caregiving        | Lower IgG Ab-response | 117  | (68)      |
| KLH         | Life events, daily stress    | Lower IgG Ab-response | 89   | (69)      |

KLH, keyhole limpet hemocyanin.
titers to tetanus vaccination (81,90,91). 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 (81), but in another study no difference was observed between elderly and young adults (92).

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

**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+ T 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 (93,94).

**Influence of immunosuppressive infections on the vaccination response.** HIV, measles virus, some bacteria (Salmonella), and helminths (Schistosoma, Nematodiridae) 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 and hepatitis A and B virus (95–98).

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 (99). Measles virus is clearly immunosuppressive. It interferes with the function of antigen-presenting cells such as monocytes and dendritic cells. This may lead to deficiencies in interleukin-12 (IL-12) production and T cell proliferation (100–102). 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 (103). The effect of chronic hepatitis B virus carriage on primary vaccinations is unknown. The same is true for helminth and bacterial infections. Salmonella, Schistosoma, and Nematodiridae may influence the function of B and T cells (104–106). Yet their influence on the vaccination response is not documented.

**Influence of nonimmunosuppressive and nonspecified 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 (107).

A number of studies have been directed to the question of whether nonspecific infections, manifested by symptoms such as diarrhea, rhinorrhea, 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 (108), whereas seven other studies reported no or only minimal clinically significant influence (109–114).

**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 crossreact or limit the replication of vaccine virus. Sabin OPV type 2, for example, interferes with the vaccination response to Sabin type 3 (115). Non-polioenteroviruses may also interfere with the vaccination response to OPV. In contrast, nonspecific enteric infection did not interfere with OPV vaccination (116).

In conclusion, although some infections may exert well-documented immunosuppressive effects in either humans or laboratory animals, their influence on the vaccination response is poorly documented. The influence of well-known immunosuppressive infections, such as measles virus and HIV, appears limited to 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 nonspecific 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.

**Discussion and Conclusion**

Vaccination titers are a reflection of the immune function, and changes in vaccination-specific immune responses are therefore considered as indicators for the effect of environmental exposures on the immune system in human populations. To date, few studies have been performed in which vaccination titers were used to detect immunotoxicity in the human population. Hence, there is as yet not much experience with sensitivity of this type of testing in epidemiologic studies. The literature to date indicates that many influences on the response to a vaccination exist, and these therefore should be taken into consideration in the design of epidemiologic studies aimed at assessing the effect of environmental exposures on which the response to vaccination is used as an indicator 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 intraperson variability in the population regarding the responses to the chosen vaccine, so that the required study group size can be determined. 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. Therefore, it is important to standardize vaccination procedures. 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 in the study design.

The data indicate that sex, 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, such as socioeconomics or culture. 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 so that it is possible to correct for these influences. It is obvious that such influences have differential relevance in different populations, such as elderly versus children or populations in wealthy societies versus underdeveloped regions.

According to the extensive animal studies reported by Luster et al. (2,3) 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 to 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 under study, it is likely that the exposed population has lower resistance to infections. 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, and so on, 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 because they only lead to more of the same symptoms. They may actually not be significant for the individual patient. But due to the high prevalence of many rather innocuous infections, such effects may, on a population basis, be significant.

With respect to the efficacy of vaccination in terms of protection, it needs to be mentioned that it is not always possible to deduce from the vaccination titers the level of protection gained. It is therefore likewise not possible to deduce from effects of environmental factors 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 (117). This is also true for postimmunization measles antibody responses and for postimmunization 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. A strong correlation exists between low antibody levels after a single dose of (measles) vaccine and high susceptibility to infection with exposure (118). So any insult to responses to these vaccines that result in titers below a certain threshold will indicate effects even for the protection at which the vaccine is aimed. It is, however, unlikely that such conditions will usually 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, even though not every individual will always be protected. This is corroborated by the findings in developing countries in which malnourishment of children results in impaired responses after vaccination, even though these alterations do not cause the general failure of vaccine strategies (though it should be mentioned that sometimes problems with vaccination to measles are associated with vitamin A deficiency). One may expect that individuals with a response to a vaccine that leads to border-line protection may be subject to experiencing a clinically significant negative consequence of diminished vaccination response if environmental exposure that affects vaccination responses occurs.

In many countries, hepatitis B vaccination is mostly done in adults, generally by three intramuscular injections of the vaccine at 0, 2, and 6 months. Specific antibody titers are generally evident after the second immunization, and maximal titers occur after the third vaccination. Vaccination to measles is mostly done in children. Infants are injected intramuscularly at the age of about 2 months and then at 14 months. For both types of vaccination, nonresponders can be observed, usually <5%. The choice for these or other types of vaccines obviously depends on the environmental factor that one wants to study, and in what type of study group. This will also determine the magnitude of the effect that is expected. In general, effects of environmental factors on vaccination titers are expected to be modest. Thus, the study group that is evaluated needs to be large enough to have sufficient power to detect such modest differences.

Groups of 1–200 individuals have been used. The nature of the environmental agent studied will also determine the design of the study, in particular at what time point antibody titers are measured. Agents that produce reversible effects require a rather high number of vaccinations, whereas persistent chemicals such as PCBs may require titers only after the vaccination procedure has been completed (10).

Vaccination titers may prove valuable tools for identifying effects of exposure to immunotoxins in the human population. However, given the many confounders, even if they are all corrected for, immunotoxins identified in animals may not induce detectable effects on vaccination titers in humans. Careful consideration of the results of experimental animal studies and epidemiologic studies is then warranted, in terms of exposure, other immune end points, and study size, to evaluate the actual risk that the immunotoxins pose to humans.

In conclusion, 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 is 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.

REFERENCES AND NOTES

1. International Programme on Chemical Safety. Principles and Methods for Assessing Direct Immunotoxicity Associated with Exposure to Chemicals. Environmental Health Criteria 180. Geneva:World Health Organization, 1996.
2. Luster MI, Porttter C, Palt DG, White KL, Gennings C, Munsen AE, Rosenfeld G. Risk assessment in immunotoxicology. I. Sensitivity and predictability of immune tests. Fundam Appl Toxicol 18:200–210 (1992).
3. Luster MI, Porter C, Palt DG, Rosenthal GJ, Germolec DR, Corsini E, Haylock BL, Pollard J, Kuch Y, Craig W, et al. Risk assessment in immunotoxicology. II. Relationship between immune and host resistance tests. Fundam Appl Toxicol 21:71–82 (1993).
4. Aarnout DL, Blye F, Kapinski K, Ferrie J MS, Triphoons H, Truelove J, McGree PF, Burns D, Tannen J, Stapley R, et al. Toxicological consequences of Arachid 125 ingestion by female rhesus (Macaca mulatta) monkeys. Part 18. Prebreeding phase: Exposure to chemicals and immunity. J Environ Health Perspect 10:811–824 (1993).
5. Yu LM, Hsin JW, Huo CC, Chan WC, Guo YL. The immunologic evaluation of the Yucheng children. Chemosphere 37:1055–1065 (1998).
6. Van Loveren H, Germolec D, Koren HS, Luster MI, Nolan C, Repetto R, Smith E, Vos J G, Vogt RG. Report of the Biathlon Symposium: advancement of epidemiological studies in assessing the human health effects of immunotoxic agents in the environment and the workplace. Biomarkers 4:135–157 (1999).
7. Kim J. Infection and cyclosporin. Rev Infect Dis 11:677–680 (1989).
8. Desjokes J, Vial T. Cytoreductive drugs. In: Immuno-toxicology and Immunopharmacology (Dean JH, Luster MI, Munsen AE, White K, eds). 2nd ed. New York:Raven Press, 1994:293–301.
9. Dewey E. Unpublished data.
10. Weisglas-Kuperus N, Patandin S, Berbers GAM, Sas TCJ, Mulder PGH, Sauer PJH, HooiKaa H. Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Amsterdam school children. Environ Health Perspect 108:1203–1207 (2000).
11. Weidlin PL, Wolf SB, Breitb AB, Appelbaum JW, Seiler CA, Mullet R, Bvem NJ, SU FM, Fer MF, Salk D. Human anti-mouse antibody suppression with cyclosporin A. Cancer 73(suppl 3):1095–1097 (1994).
12. Van Der Does-Van Den Berg A, Hermans J, Nagel J, Van Steege G. Immunity to diphtheria, pertussis tetanus and poliomyelitis in children with acute lymphocytic leukemia after cessation of chemotherapy. Pediatrics 67:222–229 (1981).
13. Gerritsen EJA, Van Tol MJD, Van’t Veer MB, Wels JMA. Antibody response after hepatitis B vaccination of Minnesota M, Osterholm MT. Risk factors for lack of detectable antibody following hepatitis B vaccination of Minnesota health care workers. JAMA 270:2935–2939 (1993).
14. Roomo A, Walsh S, Carter M, Hadler J L. Hepatitis B vaccine responsiveness in a homeless and mentally ill public safety personnel. JAMA 270:2931–2934 (1993).
15. Mancini DA, Mendoza RM, Mendoza RZ, Do Prado J A, De Andrade CM. Immune response to vaccine against influenza in smokers, non-smokers, and, in indi-
null
90. Lesourd BM. Nutrition and immunity in the elderly: modification of immune responses with nutritional treatments. Am J Clin Nutr 66:478S–484S (1997).

91. Meydiani SN, Meydani M, Blumberg JB, Leka LS, Siber G, Losewsky R, Thomson C, Pedrosa M C, Diamond RD, Stollar BD. Vitamin E supplementation and in vivo immune response in healthy elderly subjects. A randomized controlled trial. JAMA 277:1380–1386 (1997)

92. Pozzetto B, Odelin M P, Bienvenu J, Defayolle M, Aymard M. Is there a relationship between malnutrition, inflammation, and post-vaccinal antibody response to influenza viruses in the elderly? J Med Virol 41:39–43 (1993).

93. Brenan M, Zinkernagel RM. Influence of one virus infection on a second concurrent primary in vivo antiviral cytotoxic T-cell response. Infect Immun 41:470–475 (1983).

94. Kotwal GJ. Microorganisms and their interaction with microorganisms. Ann Med Int 149:351–360 (1998).

95. Tilzey AJ, Palmer SJ, Harrington C, O'Doherty MJ, Loke RH, Murray-Lyon IM, Coleman JC, Evans BA, Tilzey AJ, Palmer SJ, Harrington C, O’Doherty MJ. Influence of one virus infection on a second concurrent primary in vivo antiviral cytotoxic T-cell response. Infect Immun 41:470–475 (1983).

96. Kotwal GJ. Microorganisms and their interaction with microorganisms. Ann Med Int 149:351–360 (1998).

97. Bouchaud O, Mouis H. Vaccination and systemic diseases. Vaccinations and immunosuppression. Ann Med Int 149:351–360 (1998).

98. Loke RH, Murray-Lyon IM, Coleman J C, Evans BA, Zuckerman AJ. Diminished response to recombinant hepatitis B vaccine in homosexual men with HIV antibody: an indicator of poor prognosis. J Med Virol 31:109–111 (1990).

99. Arpadi SM, Morkowicz LE, Baughman AL, Shah K, Adam H, Wilsa A, Lambert G, Doboszycki J, Heath J L, Bellini WJ. Measles virus infection of haemophilia. Vaccine 14:1039–1041 (1996).

100. Karp CL, Wysocka M, Wahl LM, Ahearn J M, Cuomo P J, Sherry B, Trimarchi T, Groopman J. Mechanism of suppression of cell-mediated immunity by measles virus. Science 273:228–231 (1996).

101. Bell AF, Burns J B, Fujinami RS. Measles virus infection of human T cells modulates cytokine generation and IL-2 receptor alpha chain expression. Virology 232:241–247 (1997).

102. Fugler-Vivier I, Servet-Delpret C, Rivailier P, Rissooan MC, Liu Y, Rabouint-Combe C. Measles virus suppresses cell-mediated immunity by interfering with the survival and function of dendritic and T cells. J Exp Med 186:813–823 (1997).

103. Livingston BD, Alexander J, Crcmi C, Oseroff C, Celis E, Daly K, Guidotti LG, Chisani FV, Flies J, Chesnut RW, et al. Altered helper T lymphocyte function associated with chronic hepatitis B virus infection and its role in response to therapeutic vaccination in humans. J Immunol 162:3089–3095 (1999).

104. Actor JK, Shirai M, Kullberg MC, Buller RM, Sherr A, Berzoñsky JA, Meihelm infection results in decreased virus-specific CDF+ cytotoxic T-cell and Th1 cytokine responses as well as delayed virus clearance. Proc Nat Acad Sci USA 90:548–552 (1993).

105. Al-Ramadi BK, Greene J M, Meisler J J, Eisenstein TK. Immunosuppression induced by attenuated Salmonella: effect of LPS responsiveness on development of suppression. Microb Pathog 12:267–278 (1992).

106. Pritchard DI, Ali RM, Behei K J. Multiple sclerosis: clinical manifestations of human T lymphocyte function. J Exp Med 162:3088–3095 (1999).

107. Rennels MB. Influence of breast-feeding and oral poloprivus vaccines. J Infect Dis 164:S107–111 (1996).

108. Migasena S, Simasathien S, Samakoses R, Pitisuttitham A. Influence of maternal antibodies, malnutrition, and concurrent illnesses. N Eng J Med:313:544–549 (1985).

109. Edmonson M B, Davis J P, Hopfensperger DJ, Berg J L, Payton LA. Measles vaccination during the respiratory virus season and risk of vaccine failure. Pediatrics 98:905–910 (1996).

110. Rattan S, West R, Gadag V, Measles and rubella antibody response to measles in children with afebrile upper respiratory tract infection. J Pediatr 127:432–434 (1995).

111. Halsey NA, Boulou R, Mode F, Andre J , Bowman L, Yaeger RG, Toureus S, Rohde J, Boulos C. Response to measles vaccine in Haitian infants 6 to 12 months old: Influence of maternal antibodies, malnutrition, and concurrent illnesses. N Eng J Med 313:544–549 (1985).

112. Edmonson M B, Davis J P, Hopfensperger DJ, Berg J L, Payton LA. Measles vaccination during the respiratory virus season and risk of vaccine failure. Pediatrics 98:905–910 (1996).

113. Halsey NA, Boulou R, Mode F, Andre J , Bowman L, Yaeger RG, Toureus S, Rohde J, Boulos C. Response to measles vaccine in Haitian infants 6 to 12 months old: Influence of maternal antibodies, malnutrition, and concurrent illnesses. N Eng J Med 313:544–549 (1985).

114. Edmonson M B, Davis J P, Hopfensperger DJ, Berg J L, Payton LA. Measles vaccination during the respiratory virus season and risk of vaccine failure. Pediatrics 98:905–910 (1996).

115. Maldona Ya, Pena-Cruz V, de Luz Sanchez M, Logan L, Blandon S, Cantwell MF, Matsu SM, Mllan-Velasco P, Valdespino J, Seppula J. Host and viral factors affecting the decreased immunogenicity of Sabin type 3 vaccine after administration of trivalent oral polio vaccine to rural Mayan children. Infect Dis 175:545–553 (1997).

116. Triki H, Abdallah MV, Ben Aissa R, Bourabine A, Ben Ali Kacem M, Bouroudi S, Koubbar C, Zaouari S, Mohsni E, Crainic R, et al. Influence of host related factors on the antibody response to trivalent oral polio vaccine in Tunisian infants. Vaccine 15:1123–1129 (1997).

117. Hadler SC, Francis DP, Maynard J E, Thomson SE, Judson FN, Chenberg DF, Octrow DG, O’Malley PM, Penley KA, Altman NL. Long-term immunogenicity and efficacy of hepatitis B vaccine in homosexual man. N Eng J Med 24:209–219 (1986).

118. Deseda-Tous J, Cherry JD, Spencer MJ, Welliver RC, Boyer KM, Dudley J P, Zahradnik J M, Krause J, Walberg E W. Measles revaccination: persistence and degree of antibody titer by type of immune response. Am J Dis Child 132:207–209 (1978).