

Primary vaccine failure to routine vaccines: Why and what to do?

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There are 2 major factors responsible for vaccine failures, the first is vaccine-related such as failures in vaccine attenuation, vaccination regimes or administration. The other is host-related, of which host genetics, immune status, age, health or nutritional status can be associated with primary or secondary vaccine failures. The first describes the inability to respond to primary vaccination, the latter is characterized by a loss of protection after initial effectiveness. Our studies concentrate on the evaluation of immunological characteristics responsible for primary vaccine failures in different (risk) populations for which the underlying mechanisms are currently unknown. Here we summarise current knowledge and findings from our studies.

About 2–10% of healthy individuals fail to mount antibody levels to routine vaccines. Comparing the immune responses to different vaccines in non-responder and high-responder vaccinees revealed that hypo-responsiveness is antigen/vaccine-specific at the humoral but not at the cellular level. We found that T-regulatory as well as B-regulatory cells and the production of IL-10 are involved in non/hypo-responsiveness. Non-responsiveness increases with age and in particular vaccination to a novel vaccine in persons > 65 years is associated with a high low/non-responder rate, indicating that vaccine schedules and doses (at least for primary vaccination) should be adapted according to age.

In light of the growing number of allergic but also obese people, our current studies concentrate on these risk groups to reveal whether different vaccination approaches are necessary for optimal protection compared to healthy individuals. These studies are in line with the significant paradigm shift taking place in many fields of medical research and care, and will extend the concept of personalised medicine into the field of vaccinology.

Background

Vaccination recommendations and programs are based on clinical trials performed in selected, healthy and mostly young populations. The data derived from these vaccine trials on safety, immunogenicity and efficacy are thus performed under “ideal situations” aiming to reflect the normal population distribution but certainly exclude risk populations. It seems of particular importance to recognize that significant demographic changes in the population have occurred in recent decades (which will continue further). According to the UN Population Division the number of elderly people is expected to rise to 25% of the world wide population by 2050 due to advances in average life expectancy (United Nations 2002; www.un.org/esa/population/publications/wordaging19502050). This does not automatically implicate a rise in a healthy aging society. Following improvements in medical care and new therapeutic interventions, there is a continuous increase in cases of chronic disease, such as cancer, cardiovascular, metabolic or autoimmune diseases. Moreover, changes in life style and nutrition have fostered the development of “new epidemics” in developed countries, among which obesity and allergies have become huge medical issues. All these conditions are known to have distinct influences on the immune system, which may lead to an increased susceptibility to infectious diseases emphasizing the importance of effective vaccination procedures in these populations. Interestingly, very little information exists on how vaccine responsiveness is actually influenced under these circumstances and whether the existing vaccination schedules/doses are sufficient to reach optimal protection levels.

The general definition of vaccination failure is based on 2 aspects; vaccine-related and host-related factors. While inadequacies of the vaccine (such as incomplete attenuation, incorrect immunisation route or schedule, or failures in delivery due to interruption of the cold chain) are reasons for vaccine failures that can be logistically overcome, host-related factors for non-responsiveness (associated with the immune and health status, age, or genetic factors) are more difficult to define and underlying mechanisms of vaccine failure are largely unexamined or unknown.

The term non-responsiveness or primary vaccination failure is currently described by the inability of the host/vaccinee to mount sufficient protective antibody responses after primary or booster vaccination. This phenomenon affects about 2–10% of vaccinated healthy individuals^{1–4} but the immunological background, the clinical consequences, or the question of whether vaccine failure is antigen-specific or a general phenomenon are largely unknown.

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The most documented is non-responsiveness to hepatitis B vaccine in which up to 10% of otherwise immunocompetent persons do not respond with protective antibody levels to the hepatitis B surface antigen in the vaccine.¹ Due to a high number of non-responders this type of non-responsiveness has been more closely investigated. Risk factors such as obesity, heavy smoking or chronic renal failure have been described in addition to genetic predisposition in certain HLA class II haplotype (HLA-DRB1, HLA-DQB1).⁵⁻⁷ Genetic predisposition to vaccine failure has also been described for other vaccines such as influenza.⁸ A much lower but significant number of non-responders was found among hepatitis A vaccinees in an unselected patient collective of travelers, of whom 2% were characterized by a lack of specific antibodies after primary vaccination.² Also in individuals vaccinated against tick-borne encephalitis (a flavivirus infection of high prevalence in Northern and Central Europe) a non-responder rate of about 5% was recently identified.⁹ Notably, these low/no-responders were most frequently found in the age group >50 y indicating that immunosenescence contributes to this type of non-responsiveness.¹⁰ It is well recognized that age related immunosenescence contributes to the increased susceptibility of the elderly to infectious disease and to the poor outcome of vaccination.¹¹ The reduced responsiveness of the aged immune system is responsible for both increased susceptibility toward infectious and pathological events and suboptimal responsiveness to vaccination. Optimising vaccination strategies and vaccines for the elderly is consequently an important task, unfortunately the current understanding of the mechanistic basis of immunosenescence is however still partial. Similarly, in diseases or specific therapeutic interventions that lead to immunosuppression the non-responder rates and underlying mechanisms of vaccine failure have hardly been investigated.¹²⁻¹⁴ In these populations vaccination strategies have been exclusively based on empiric and/or theoretical considerations leading to insecure protection rates.

We therefore have focused our research on the in depth characterization and evaluation of humoral and cellular immune responses to a variety of vaccines in different risk populations with the following questions of interest:

1. Is low/non-responsiveness a general or antigen-specific phenomenon?
2. Is there a correlation between humoral and cellular immune responses in non-responders?
3. Are there characteristic changes of cellular parameters in different types of non-responders?
4. What are the characteristics and underlying mechanisms of altered vaccine responsiveness in certain risk situations (e.g. age, allergy, obesity)?
5. What are the consequences for vaccination schedules, recommendations and vaccine design?

Non-Responsiveness to Routine Vaccines in Immunocompetent Vaccinees

Evaluation of seroprotection following vaccination is based on the measurement of specific antibody titer. The absence of

antibodies can however not distinguish between individuals whose antibody levels had declined since primary vaccination and those which remain undetectable due to an intrinsic inability to sufficiently respond to the vaccine. The current practise for identification of “real” non-responder is based on an additional booster vaccination. The lack of antibody responses even after booster vaccination does however not automatically mean lack of protection and increased susceptibility to clinically significant disease. At least in hepatitis B vaccinees that fail to generate an anamnestic antibody response upon booster vaccination no cases of acute hepatitis B or chronic antigen carriage have been reported.¹⁵⁻¹⁷ This has been explained by the fact that protective immunity is achieved by a complex interplay between naïve and memory B and T cells, in which antigen-specific memory T cells detectable also in the blood of seronegative individuals are most likely able to render anamnestic responses. However, the immunological interactions between the different cell populations have been rarely investigated in responder and non-responder vaccinees.

In order to investigate whether there is a correlation of specific humoral and cellular vaccine responses we first investigated a small number of “real” non-responder to hepatitis A after booster vaccination in comparison to high-responders and sought to identify prediction markers that, independently of antibody measurements prior and after booster vaccination, help to identify individuals that are likely to fail to respond.¹⁸ In this study we observed that cytokine concentrations (IL-2, IFN- γ and IL-10) derived after antigen-specific stimulation of peripheral blood mononuclear cells (PBMC) were high in vaccinees with high antibody levels but low to undetectable in individuals lacking hepatitis A specific antibodies, indicating a clear correlation between vaccine specific humoral and cellular responses. Non-responsiveness was further associated with a significantly higher percentage of regulatory T cells as well as a minimal or no expression of the hepatitis A receptor on CD4 + T cells, indicating a possible role as prognostic/predictive marker of non-responsiveness to hepatitis A, which needs further investigation in prospective trials.

The question of whether non-responsiveness is an antigen-specific event or may occur in the same individual to several vaccine antigens was of further interest to be investigated. Non-responders to tick borne encephalitis (TBE) or hepatitis B antigen with a history of previous TBE vaccinations were selected and booster-vaccinated with TBE as well as influenza-vaccine.¹⁹ In TBE non-responders low to undetectable pre-vaccination TBE-titres remained low after booster vaccination but sufficient influenza-antibodies were detected. There was a positive correlation of humoral and cellular immune responses, namely low TBE-titres were associated with a lack of antigen-specific T-cell responses and responses to influenza were robust in terms of antibodies and cytokine production. In contrast, in hepatitis B non-responders (vaccinated against TBE and influenza) sufficient humoral responses to both antigens were induced despite lacking antigen-specific IL-2 and IFN- γ production. Importantly, these patients showed high IL-10 base-line levels *in-vitro*. While in TBE non-responder no HLA correlation but an age dependent

influence on vaccine failure was found, the HLA-DR subtypes HLA-DRB1 and HLA-DQB1 were overrepresented in the hepatitis B non-responder group and linked to high IL-10 base line levels. Both non-responder groups had increased IL-10 producing FOXP3⁺ T-regulatory cells after vaccination. However, only in hepatitis B non-responders - showing also elevated pre-vaccination IL-10 levels - a prominent population of B-regulatory cells was detected. Thus, our data showed that TBE non-responsiveness was strictly antigen-specific, but in hepatitis B non-responders there was a dichotomy of unimpaired vaccine specific humoral and impaired cellular responses. We concluded that the immunological pathways of non-responsiveness follow different patterns depending both on the vaccine antigen and the genetic predisposition of the vaccinees.¹⁹ At this point the clinical consequences, namely whether these individuals are at higher risk to acquire infections/disease cannot be answered, but surveillance must continue with studies to better understand the mechanism of protection in these individuals.

Influence of Age on Vaccine Responsiveness

Aging is associated with a decline in immunological functions leading to a state of immunosenescence.^{11,20} The changes include a decline in the B- and T cell repertoire along with a decrease of the naïve cell pool, while the memory and terminally differentiated T effector cells of restricted diversity increase. Part of this transformation has been associated with cytomegalovirus (CMV) infection.²¹⁻²³ As a consequence of this immune remodelling elderly people are more susceptible to infectious diseases²⁴ and at the same time vaccination in the elderly is less immunogenic and hence less effective.²⁵ For booster vaccinations it has been shown that the period of protection is reduced with generally lower post-booster antibody concentrations due to a more rapid decline of protective antibody levels compared to young vaccinees.²⁶ It was shown recently, however, that a decline in the quantity of antibody levels does not necessarily correlate with a decline in the antibody quality in the elderly; no age-related differences in the antibody avidity or neutralising activity of antibodies was found compared to young controls.²⁷ Response rates to booster vaccination in the elderly may further depend on the type of vaccine being used for primary vaccination, as long lasting protection and good responsiveness to boosting has been described after exposure to live vaccines, such as polio, earlier in life.²⁸

With respect to primary vaccination in the elderly, data on the immunogenicity are scarce, the few existing datasets confirm lower antibody concentrations compared to younger vaccine recipients.^{27,29} In particular, to what extent cellular responses to primary vaccination are affected by age and concomitant CMV infection has not been described so far. We therefore performed a study to determine age-associated differences of humoral and cellular immune responses upon primary vaccination with a neo-antigen. We chose to study primary responses to inactivated Japanese encephalitis (JE) vaccine as it can be assumed that the included subjects have not been previously exposed to this antigen/virus that is endemically restricted to parts of the Asian

continent. Comparing JE-specific antibody responses between elderly (mean 68 years) and young vaccinees (mean 24 years) revealed significantly lower antibody titres in the elderly with more than 40% not (or hardly) responding to JE-vaccination. The reduced humoral immune responses in the elderly were associated with reduced cytokine production of *in vitro* stimulated PMBCs along with a significant increase of T regulatory cells. Additionally, higher frequencies of late-differentiated effector and effector memory cells, while lower percentages of early differentiated and naïve CD4⁺ and CD8⁺ cells were detected among the elderly vaccinees. CMV has been described as major driver of immunosenescence, the majority of elderly subjects were seropositive for CMV which correlated with the reduced antibody titres and increased late differentiated CD8⁺ and CD4⁺ T cells. Recently a novel CMV-induced regulatory CD4⁺ T cell subset has been described in CMV-infected people.³⁰ Whether the increased T regulatory cells described in our study are also CMV induced and responsible for the described response failure is currently under investigation.³¹ Our data suggest that primary vaccination with a neo-antigen should preferentially be applied at younger age (<50 years) to ensure sufficient and long lasting responsiveness. To improve immune responses in cases where primary vaccination is indicated in elderly (>60 years) accelerated schedules, higher doses or vaccines including immune-enhancing adjuvants need to be considered and more data generated.²⁴

Vaccine Responsiveness in Risk Populations (e.g., Allergic and Obese Individuals)

Atopic/allergic disease is characterized by an immunological hyper-responsiveness to allergens along with a general shift toward Th2 responses. During causal treatment with specific immunotherapy (SIT) immunosuppressive mechanisms are induced via counter-regulatory Th1 cells, T regulatory cells and IL-10.³² Whether allergic individuals, and particularly those who undergo immunosuppressive immunotherapy, display altered responsiveness to routine vaccines has rarely been investigated. Studies in atopic children who were vaccinated against tetanus or pertussis did not show significant differences in antibody levels compared to healthy children.³³ Analysis of the postnatal maturation of T-helper cell responses to several antigens including tetanus toxoid showed a continuation of Th2-biased immune responses but decreased capacity for production of Th1 cytokines (INF- γ) compared to healthy children.³⁴ In a recent study in adults evaluating seroimmunity against TBE 10 y after booster vaccination, a subgroups of allergic individuals reporting chronic or seasonal recurrent allergic disease against inhalant, food or contact allergens was evaluated regarding the humoral vaccine responses. Surprisingly, these individuals displayed significantly higher TBE-specific antibodies compared to persons without any allergy. The increased antibody titres might be a result of the generally increased Th2-biased hyper-responsiveness, but at the same time do not implicate increased quality and functional capacity, as avidity testing was not performed.¹⁰ We therefore continued a

study with allergic patients, also including patients undergoing specific immunotherapy, for detailed analysis of humoral and cellular responsiveness upon TBE vaccination. Preliminary results confirm that antibody responses are increased in allergic individuals but the humoral responses do not correlate with the cytokine production profile of antigen-restimulated PBMCs. The qualitative analysis of the antibody responses is currently under investigation, which is of particular interest in patients undergoing SIT where a significant increase of regulatory T cells was found. We anticipate that the final results from this study will help to increase our understanding of vaccine responsiveness in allergic individuals and whether adapted vaccination schedules are required for these individuals.³⁵

Similar open questions exist for obesity, a metabolic disorder that has also become a considerable health concern in many countries affecting an alarmingly high number of adults but also children and adolescents. Apart from the known secondary diseases related to being overweight, such as cardiac disease, type II diabetes, certain types of cancer or osteoarthritis, recent studies have shown that obesity causes a state of low-grade inflammation, and has direct effects on the immune system leading to immunosuppression.^{36,37} Accordingly, obese people have been shown to suffer from an increased susceptibility to several infections, such as influenza, tuberculosis or pneumococcal diseases. The mechanisms underlying these immunosuppressive effects are not fully understood but seem to be triggered by the white fat tissue constituting the major part of fat tissues, which acts as an active endocrine organ producing different cytokines and adipokines (e.g. leptins) and thereby interacting with respective receptors expressed on T and B cells.³⁸ In light of the burden of obesity to infectious diseases an adequate protection by vaccination is of particular importance. Generally very few vaccination studies exist in obese people, but it has been documented for some vaccines that an elevated body mass index (BMI) is associated with poor vaccine responsiveness. A study in health care workers having received hepatitis B vaccination showed that the non-responder rate of people with a BMI > 25 was significantly higher compared to persons with a BMI <25.³⁹ Similar findings were recently surveyed in women vaccinated against hepatitis B,³⁷ as well as in a cohort of travelers vaccinated against hepatitis A.² The negative impact of obesity on vaccine immune outcomes has also been shown for seasonal influenza vaccines⁴⁰ and for

tetanus toxoid vaccine in children.⁴¹ In order to gain more insight in the humoral and cellular responsiveness upon vaccination as well as underlying mechanisms behind non-responsiveness we are currently performing a vaccination trial with TBE vaccine in obese adolescents and adults. This study will hopefully increase our understanding of the complex interplay between obesity, inflammation and vaccine immunogenicity and help to clarify whether more sufficient strategies, such as change of vaccine doses and booster intervals are necessary to ensure sufficient protection of this growing risk population.

Consequences and Conclusions

Current vaccination recommendations, schedules and vaccine design have largely neglected the growing number of risk populations that for different reasons present an impairment or modulation of immunological functions; with increased infection susceptibility and impaired vaccine responsiveness. A better understanding and increased evaluation of the distinct mechanisms of immunological impairment is needed to allow a more personalised approach to vaccine strategies and vaccine design in different risk groups to guarantee higher vaccine responsiveness. Risk group targeting approaches may engage several steps, such as the development of new vaccine formulations including adjuvants targeting both the innate and adaptive arm of the immune system, the introduction of higher doses of vaccines, or the application of accelerated schedules and more frequent booster doses. A change in the immunisation route (e.g., from intramuscular to intradermal), as shown with the positive response in the elderly with intradermal influenza vaccine, may also apply to other vaccines in risk groups. Finally, the identification and characterization of predictive markers of non-responsiveness and establishment of algorithms based on pre-existing inflammatory and immunosuppressive factors may help in the decision making process for those who will particularly benefit from modified vaccination strategies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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