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## Towards strengthening memory immunity in the ageing population

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# GENERAL DISCUSSION





## Summary of the main findings

Timely vaccination of middle-aged adults may be an alternative strategy to increase the memory immunity against infectious pathogens before reaching old age. This thesis is focussed on providing knowledge on the immunological fitness of middle-aged adults as an interesting target group for future vaccine interventions in the rapidly ageing population.

## The immune phenotype of middle-aged adults and the influence of sex and chronic CMV infection

Immunological ageing has profound effects on the immune phenotype, in both the innate and adaptive compartments. Primarily, the aged immune phenotype is characterized by a shift from naïve T-cells to memory T-cells, consequently leading to a reduced TCR repertoire [1-3]. Chronic CMV infection is known to accelerate the accumulation of late-differentiated T-cells, although the interaction with sex has been given little attention [4-7]. In **Chapter 2** of this thesis, we describe that both sex and chronic CMV infection individually and synergistically affect the immune phenotype of middle-aged adults, which is based on an extensive enumeration of the absolute numbers of both innate and adaptive immune cells in the circulation. Male sex is associated with lower absolute numbers of cells in the majority of the immune cell subsets, whereas both male and female CMV seropositive persons indeed possess higher numbers of late-differentiated T-cells. Moreover, analysis of the interaction between sex and CMV infection suggests an accelerated immune ageing phenotype in CMV infected males as compared to CMV infected females.

## Primary vaccination in middle-aged adults

As a consequence of immune ageing, responses to *de novo* pathogens may be severely impaired. Therefore, it is suggested that immunizations against *de novo* antigens will have to be established before reaching old age [8-10]. In **Chapter 3**, we show that a primary meningococcal vaccination, towards which no or very low pre-vaccination immunity exists, is highly immunogenic in middle-aged adults.

This tetravalent meningococcal (MenACWY-TT) vaccination induced naïve immune responses in the majority of the middle-aged adults to meningococci groups W (MenW) and Y (MenY), contrary to recall responses to meningococci C (MenC) (**Chapter 3**). Besides this robust immunogenicity, early signs of immune ageing are already visible in the middle-aged adults (**Chapter 4**), since this age group showed lower functional antibody titers as compared to adolescents who received the same primary vaccination. These differences were mainly explained by a sharp reduction of antigen-specific IgM responses in the middle-aged adults. In **Chapter 3**, these IgM responses were found essential in the antibody functionality (bactericidal activity) directed to the meningococcal groups W

and Y. This decline in IgM responses with advancing age may be extrapolated to other bacterial infections and vaccinations, such as pneumococcal conjugate vaccination, for which functional antibody titers strongly declined between 65 and 80 years of age [11]. Consequently, these results strengthen the recommendation of early vaccination, in middle-aged adults, against *de novo* (bacterial) antigens.

In addition, we observed a mixed TT-specific Th1, Th2 and Th17 response towards the TT-carrier protein included as a conjugate carrier in the vaccine. Our results also imply enhancement of circulating follicular helper T-cell responses after vaccination of middle-aged adults (**Chapter 5**). These results suggest the induction of effective T-cell responses in order to provide help to B-cells in response to bacterial polysaccharides. Remarkably, these TT-specific T-cell responses did not directly correlate with the meningococcal antibody responses. Of importance, even decades after previous TT vaccinations, high levels of pre-vaccination TT-specific T-cells were found in part of the middle-aged adults. These high TT-specific pre-vaccination T-cell responses were positively correlated with the follicular helper T-cell response to the TT-carrier, underlining the importance of maintaining TT-specific memory immunity throughout the lifespan.

### **VZV booster vaccination in middle-aged adults**

Next to the administration of primary immunizations, middle-aged adults are especially of interest for timely varicella zoster (VZV) vaccination, ensuring protection at an older age. As a consequence of immunological ageing, the VZV-specific cellular immunity (CMI) after natural exposure has waned. Other studies previously reported that this VZV-specific CMI is insufficiently boosted by Zostavax vaccination in the elderly in order to prevent herpes zoster disease [12-14]. Consequently, alternative solutions to decrease the herpes zoster disease burden in the elderly are under evaluation. Currently, the vaccine effectivity of a herpes zoster subunit vaccine is being studied in clinical trials and shows promising results in the elderly [15-17]. These data imply differences in responsivity to Zostavax and the (candidate) subunit vaccine in elderly persons. Therefore, it will be important to understand the quantity and quality of the VZV-specific immune responses upon vaccination in more detail and to determine factors affecting these responses in the target population(s) to improve VZV vaccination schedules in older adults.

In **Chapter 6**, we show that VZV (Zostavax) vaccination of middle-aged adults results in short-term enhancement of the VZV-specific CMI. Of importance, we are the first showing an age-dependent effect of pre-vaccination VZV-specific CMI on vaccine immunogenicity. In short, our findings suggest that participants in their early sixties, who often possess low VZV-specific CMI, benefit most from timely VZV vaccination, at least up till one year. In contrast, the vaccine might 'exhaust' the VZV-specific T-cell pool in participants who already have substantial VZV-specific CMI, a notion that warrants further investigation. In addition,

the presence of low VZV-specific CMI may be associated with a high CD4/CD8 T-cell ratio and consequently a high CD4/CD8 T-cell ratio might identify middle-aged adults eligible for early VZV-vaccination.

### **Biomarkers for vaccine responsiveness**

In order to ensure that effective precautions are taken before reaching old age, predictive biomarkers for vaccine responsiveness in older adults are highly warranted. Currently, the discovery and validation of these markers is challenging. The explorative biomarker study as represented in **Chapter 7** provides a basis for the determination of potential biomarkers for primary meningococcal vaccine responsiveness in middle-aged adults. In this chapter, we show that low and high vaccine responders significantly differed in their CD4 T-cell signature. The low vaccine responders were characterized by high absolute numbers of naïve and memory Treg cells, as well as high numbers of CD4 naïve T-cell subsets, including the CD45RACD25<sup>dim</sup> naïve cells.

### **Implications and remaining questions**

#### ***Is immunological ageing accelerated in CMV infected males?***

The differential effect of CMV carriage on the immune phenotype of middle-aged males and females, as presented in **Chapter 2**, suggests accelerated immune ageing in CMV infected males. This differential effect of CMV carriage on immune phenotype likely originates from hormonal influences, since testosterone is generally considered immune suppressive whereas levels of estrogens generally stimulate immune function [18, 19]. With respect to hormonal fluctuations throughout the lifespan, the interactive effects of CMV and sex on the immune phenotype as well as the clinical implications of these differences remain to be clarified in different age groups. Recently, interactive effects of CMV infection and sex were found absent in young children [20].

Besides the suspicion of accelerated immune ageing in CMV infected middle-aged males, meningococcal vaccine responses in these males are comparable to both CMV+ females, as well as CMV- males. Importantly, the vaccine response to the meningococcal vaccine is mostly B-cell mediated and hence effects of CMV infection, mostly affecting T-cells, may be limited. On the other hand, effects of CMV seropositivity may be present on primarily T-cell mediated vaccine responses, such as influenza or VZV vaccination, although this currently remains controversial [21-23]. Well powered studies are needed to clarify this issue. Consequently, the notion of accelerated immune ageing in CMV infected middle-aged males requires additional investigation. Moreover, differences between sexes in immune responses to vaccines and infectious diseases in general, as well as the clinical consequences of these differences, need to be further elucidated [24].

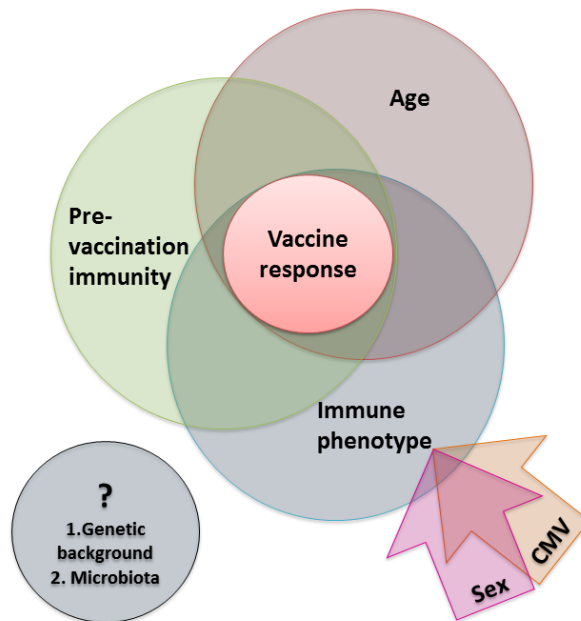
***What are the prospects on using predictive biomarkers for vaccine responsiveness in older adults?***

It would be beneficial to identify biomarkers predictive of infection risk and vaccination responsiveness in ageing individuals over time to allow the development of successful vaccination schemes for the ageing population. Consequently, the discovery of predictive biomarkers for vaccine responsiveness in older adults is focus of current research [10, 23, 25-31]. Due to the complex interactions between heritable and non-heritable factors in the immune response to vaccinations, the discovery of universal predictive biomarkers for vaccine responsiveness is a challenge and needs comprehensive investigation. Also the highly diverse immune phenotypes, also called 'immunotypes' in relatively healthy individuals complicates (universal) biomarker research [32].

Several candidate biomarkers, based on the pre-vaccination immune phenotype, pathogen-specific pre-vaccination immunity and sex have been discovered in the studies described in this thesis as well as by others. In the explorative biomarker study described in **Chapter 7** of this thesis, we found that a CD4 T-cell signature, consisting of high numbers of CD4 naïve, post-thymically expanded CD45RA+CD25<sup>dim</sup> cells as well as naïve and memory Treg cells, was associated with low vaccine responsiveness towards a primary meningococcal vaccine. These data confirm the previously reported negative associations between high numbers of circulating Treg cells and vaccine responses in older persons [23, 33]. The negative association between high numbers of naïve CD4 T-cells and vaccine responsiveness is surprising, since, except from the oldest olds [34], a broad and functional naïve repertoire is generally accepted as beneficial at older age [35, 36]. Accordingly, these findings require additional investigation.

As also previously described by others [28, 30], the pathogen-specific pre-vaccination immunity was found to affect the vaccine responses described in this thesis (**Chapter 3 and 6**). Since quantification of this pathogen-specific pre-vaccination immunity before vaccine administration is labour intensive, predictive factors for the presence of (low) pre-vaccination immunity are warranted. For instance, the amount of natural contacts with the varicella zoster virus might be predictive for the level of pre-vaccination VZV-specific CMI. Consequently, factors such as exposure to children with chickenpox could be used to define risk groups eligible for vaccination.

Based on the findings described in this thesis, we propose that a combination of age, the pre-vaccination immune phenotype (in particular the CD4 T-cell signature), and the pathogen-specific pre-vaccination immunity predict vaccine responsiveness in adults (**Figure 1**). Sex and infection with CMV were found to alter the immune phenotype, but no direct interaction with vaccine responsiveness was observed. Notwithstanding, genetic background and the microbiota composition were not taken into account, which could have added to the predictive factors in our analysis.



**Figure 1. The combined effect of age, pathogen-specific pre-vaccination immunity and the pre-vaccination immune phenotype in predicting the vaccine responses in adults.**

Pathogen-specific pre-vaccination immunity is depicted here as pre-vaccination immunity, and the pre-vaccination immune phenotype as immune phenotype. Sex and CMV were found to impact the immune phenotype, but were not directly related to the vaccine responses. Effects of genetic background and microbiota were not taken into account in this thesis.

Large cohort studies, applying standardized multivariate approaches and investigating diverse vaccine responses, are required to validate these candidate biomarkers. Hence systems vaccinology, a derivative of systems biology [37, 38], combining data on genetic background, microbiome composition, and environmental factors such as diet, stress and infections, is a promising tool to reveal underlying networks of immune responses to vaccination. These analyses may subsequently add to the identification of those who will, or will not benefit from vaccination [32, 39]. Till now, systems vaccinology revealed molecular signatures in blood associated with the strength of the immune response induced by yellow fever vaccination. In addition, signatures of effective influenza vaccine responses are beginning to be uncovered [39]. Noteworthy, these predictive signatures were not comparable between the different vaccines, questioning the discovery of universal predictive biomarkers for vaccine responsiveness [39]. As a result, more research is needed to clarify the use of universal predictive biomarkers for vaccine responsiveness in older adults.



### ***Future life-long vaccination programs: age-based or personalized?***

Knowledge on pathogen-specific vaccine responsiveness is needed in different age groups and groups with different demographic characteristics in order to substantiate life-long vaccination schedules. Currently, vaccination programs for babies and children in the national immunization program are based on chronological age, which works fine for young age groups. In addition, also HPV and influenza vaccinations are advised in an age-based manner to teenage girls and older adults, respectively. Although age-based vaccination programs might be the easiest and most feasible option, this might not be true for those programs in older age groups, due to the highly diverse immune health. Yet, some vaccinations, like diphtheria and tetanus, might benefit from an age-based approach, since repeated vaccinations are advised every 10 years. Likewise, vaccinations against novel pathogens, posing a substantial risk for the elderly, might be preferred in an age-based approach, classifying middle-aged adults as an interesting target group (**this thesis**).

Certain vaccines may benefit from personalized vaccination schedules, as also suggested by others [38]. An example is the varicella zoster vaccination, because of the highly variable pre-vaccination immunity in middle-aged adults that was shown to affect the vaccine responses (**Chapter 6**). Personalized vaccination demands robust and reliable predictive markers to identify persons eligible for vaccination. As mentioned above, the discovery of these predictive markers is highly challenging. In addition, pre-vaccination screening of older adults before vaccine administration is labour intensive and entails high screening costs. Subsequently, the feasibility of these personalized vaccination approaches remains to be elucidated, but dedicated studies such as described in this thesis and the use of immune phenotyping in combination with systems vaccinology are promising to identify 'immunotypes' eligible for vaccination [38]. This personalized approach might enable the implementation of vaccination schedules based on risk groups, applying different timing, doses, and adjuvants in order to ensure protection in these vulnerable 'immunotypes'. Moreover, if these vulnerable 'immunotypes' could be determined using measures such as chronic disease (history), sex, living environment, profession, social economic status etc., life-long vaccination programs based on predefined immunological risk groups seem within our reach. Importantly, these targeted vaccination programs may improve the protective outcomes leading to lower medical costs and subsequently refund the high cost of pre-vaccination screening to allow personalized or group based vaccination.

### ***What are the benefits of vaccination at middle-age on the immunological memory of the elderly?***

The beneficial effects of timely vaccination in middle-aged adults until high age are currently unexplored and require further research. In an attempt to predict the long-term immunogenicity of the primary conjugated meningococcal vaccination, we employed

bi-exponential decay modelling (**Chapter 3**). Using this model it is estimated that approximately half of the participants will have protective antibody titers 10 years post-vaccination, a notion that needs confirmation by additional sampling of the participants at this time point. Historically, meningococcal disease in the elderly has been low. For this reason, the meningococcal vaccine was mainly employed in this study as a model vaccine for primary vaccination due to the minimal interference of pre-vaccination immunity. However, an outbreak of MenW is currently ongoing in the Netherlands, affecting people from all age groups including the elderly [40, 41]. Accordingly, our study co-incidentally provides very relevant information in the recent discussion of vaccine protection of the elderly against meningococcal disease.

Importantly, participants in their early sixties showed a slightly lower antibody response as compared to participants in their early fifties. Yet, the long-term impact of this small difference in antibody titres has to be investigated. Moreover, the findings of accelerated antibody decay in the middle-aged adults as compared to the adolescents (**Chapter 4**), suggest an age related reduction in the formation and survival of long-lived plasma cells in the bone marrow [42, 43]. The decline in bone marrow niches for long-lived plasma cells and subsequent reduced maintenance of these plasma cells is likely caused by age-dependent increases in fat deposition and reduced production of survival factors in the bone marrow [42, 44-46]. Accordingly, investigation of the formation and maintenance of long-lived plasma cells in the middle-aged adults is of interest to understand the persistence of long-term antibody responses in older adults. However, bone marrow samples of healthy adults are difficult to obtain. In addition, investigating the formation and maintenance of memory B-cells after primary or even booster vaccination in middle-aged adults is essential to understand the long-term benefits of early vaccinations for elderly persons. Sufficient numbers of functional long-lived memory B-cells will presumably positively influence immune responses towards secondary contacts at older age. Of importance, these memory B-cells might be present independently of protective antibody titers, as previously shown [42, 47, 48]. Correspondingly, the long-term effects of early VZV vaccination in middle-aged adults are unknown and also require additional investigation. Essential information could be obtained by comparison of the VZV-specific immunity between elderly persons that received early vaccination and those who did not, or received the vaccination at an older age. Remarkably, the results as presented in **Chapter 6** suggest possible exhaustion of the VZV-specific peripheral T-cell pool, based on enhanced numbers of VZV-specific late-differentiated CD8 T-cells, after vaccination in participants with high levels of pre-vaccination CMI. These participants and the maintenance of the VZV-specific T-cells over time in these participants have to be closely monitored in order to clarify this notion. In addition, measurement of the VZV-specific immune responses in tissues, i.e. the skin, will enhance our understanding on the VZV-specific T-cell responses in middle-aged adults. Importantly, the benefits from

early VZV vaccination are likely to be reduced in the Dutch population, where all children are infected with VZV at a relatively low age and high natural circulation of the virus is present [49]. In contrast, populations being vaccinated during childhood and lacking VZV circulation environments probably need to be revaccinated at a younger age [50, 51].

Importantly, risks for immune hypo or hyper responsiveness after repeated vaccinations throughout the lifespan, as previously shown for certain vaccines [52], have to be carefully investigated. Consequently, thorough pathogen-specific research has to determine the benefits of early vaccination on the immunological memory of the elderly.

### ***Are middle-aged adults willing to receive vaccinations, before reaching old age?***

In view of the increasing concerns on vaccination in the society and the declining vaccination coverage [53, 54], investigating and increasing the willingness of middle-aged adults to receive vaccination before reaching old age is a critical step towards life-long vaccination programs. In order to improve the herd immunity, vaccine programs for middle-aged adults can only be beneficial when a large part of the middle-aged adults will accept these vaccinations.

Previous research nicely showed that adults above 50 years of age apply multiple arguments in the decision to accept vaccinations, of which the vulnerability to the infectious disease and usefulness of the vaccination at older age seems most prominent [55]. Moreover, in contrast to adults above 65 years age, also the number of total required vaccinations was a point of attention [56]. Overall, the highest acceptance rates in adults between 50 and 65 years of age was observed for pneumococcal vaccination, exceeding the rates for herpes zoster, pertussis, and influenza vaccination [56]. Noteworthy, the willingness of the Dutch middle-aged adults to participate in the studies described in this thesis was high and exceeded our expectations.

Consequently, reliable predictions on the vulnerability of future elderly towards infectious diseases as well as knowledge on the long-term beneficial effects of early vaccinations are needed to motivate middle-aged adults to receive vaccinations before reaching old age. Of importance, communication of these results, for example using social media, to the public in easily understandable language is crucial to enhance the vaccination acceptance in older adults.

### ***Will there be a role for rejuvenation medication in future vaccination programs for older adults?***

Currently, a lot of research is conducted towards the development of medicines and compounds that rejuvenate the ageing immune system. Several promising discoveries have been done and are under investigation for humane use. First of all, CASIN, a compound inhibiting important regulators in cell cycling, is found to rejuvenate hematopoietic stems

cells in old mice and subsequently improves vaccine responses [57]. Also, targeting of the FOXO4/P53 pathway may remove senescent cells and correspondingly rejuvenate surrounding tissues [58, 59]. It remains to be established, however, if the manipulation of these targets, so far yielding promising results in mice, can be successfully translated to rejuvenation strategies in humans.

In humans, inhibition of mTOR, the master regulator of cell growth and metabolism, improves vaccine responses to influenza in older adults [60], whereas the blockade of sestrins, stress molecules, restores functions of senescent T-cells and CMV and VZV specific T-cell responses [61].

For these outstanding findings, research on optimal doses and treatment strategies are crucial and are still under investigation. For example, the use of rapamycin, an mTOR inhibitor has important side effects and is unlikely to reach successful clinical development as anti-ageing therapy [62]. Crucial in this discussion on the use of rejuvenation medication is the central question whether drugs should be administered to seemingly healthy participants [59, 62]. Notwithstanding, these developments are exciting and will certainly impact vaccination programs if vaccine responsiveness in the elderly can be safely improved.

## Future perspectives

After showing robust vaccine responses in the middle-aged adults (this thesis), future research should focus on the beneficial effects of these early vaccinations on the memory immunity up to high age. These results are essential for the development of life-long vaccination programs in the rapidly ageing population. Part of this information could be gathered by additional sampling of the study participants described in this thesis approximately 10 years (or longer) post-vaccination. Preferably, the meningococcal and VZV specific immunity in these participants should be compared to age-matched controls not having received the early vaccination. If possible, determination of memory B-cell frequencies in the meningococcal vaccinated participants will enhance our understanding on memory formation and maintenance in older adults.

In addition, it would be useful to compare the incidence of herpes zoster disease in a large cohort of elderly persons, in which half of the participants received early VZV vaccination at middle-age and the other half did not. Moreover, effects of natural VZV virus circulation on the maintenance of VZV-specific memory immunity over the lifespan, as well as the notion of immune exhaustion by additional VZV vaccination in participants with high pre-vaccination immunity have to be evaluated. Of note, important developments are ongoing surrounding a new adjuvanted varicella zoster subunit vaccine, that shows promising results on vaccine effectivity in the elderly [63]. Comparison of the vaccine responses induced by Zostavax and the subunit vaccination are useful to improve our understanding of VZV specific immune responses in older adults.

In view of the increasing life-expectancy, future studies may also investigate the effectiveness of additional booster vaccinations at high age after initial vaccination in the middle-aged adults.

Moreover, the discovery of predictive biomarkers may help to identify groups eligible for vaccination. In order to clarify the use of universal predictive biomarkers for vaccine responsiveness, future human clinical trials should be performed using different vaccines, different age groups, large cohorts and similar systems vaccinology approaches.

Accordingly, pathogen-specific life-long vaccination programs have to be carefully implemented, also taking into account the risks to induce hypo responsiveness or undesired hyper responsiveness after repeated vaccinations. These life-long vaccination programs should be substantiated by in depth epidemiological research forecasting the burden of infectious diseases in the future elderly, including predictions of the major infectious pathogens responsible for infections in the elderly.

Finally, enhancement of the public trust in science as well as investment in the public awareness of the risks accompanying the rapidly ageing of the population is essential to increase the willingness of middle-aged and older adults to accept vaccinations.

### **Concluding remark**

In search of healthy ageing, middle-aged adults are a target group of interest to receive timely vaccinations before reaching old age. The results described in this thesis enhance our understanding of the immunological fitness of middle-aged adults and subsequently bring us closer to vaccination programs that are adapted to the rapidly ageing population.

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