Biology of Immune Responses to Vaccines in the Elderly

Birgit Weinberger, Dietmar Herndler-Brandstetter, Angelika Schwanninger, Daniela Weiskopf, Beatrix Grubeck-Loebenstein#

Immunology Division, Institute for Biomedical Aging Research, Austrian Academy of Sciences, Innsbruck, Austria

Address correspondence and reprint requests to:

Prof. Beatrix Grubeck-Loebenstein

Institute for Biomedical Aging Research

Rennweg 10, 6020 Innsbruck, Austria

Telephone: +43-512-583919-10; facsimile: +43-512-583919-8

Email address: beatrix.grubeck-loebenstein@oeaw.ac.at

Key words: aging, vaccination, innate immune response, adaptive immune response, immunosenescence

Running title: Immune responses to vaccines in old age

Abstract

With increasing age the human immune system undergoes characteristic changes termed immunosenescence, which lead to increased incidence and severity of infectious diseases and to insufficient protection following vaccination. Functional defects and altered frequencies of innate and adaptive immune cells impair local responses at the site of vaccine injection, hamper the generation of primary responses to neo-antigens, prevent the effective induction of memory lymphocytes and decrease the effect of booster vaccination. As a result, antibody responses of elderly vaccinees are weaker and decline faster, and long-term protective effects of vaccination cannot be taken for granted in the elderly. Improved vaccination strategies, new adjuvants and new vaccines that specifically target the aged immune system will help to overcome the limitations of immunosenescence and ensure a better protection of the vulnerable elderly population.

Within the last decades, progresses in health care, the advent of antibiotics and vaccination and improved life-standards have led to a dramatically increased life-span. The demographic changes associated with these developments challenge the health care and social systems of all developed countries. It has been predicted that by 2050 almost 40% of the European and US-population will be older than 60 years [1]. With this perspective, public and scientific interest in age-related diseases and strategies to improve the quality of life of the elderly is continuously growing.

One major health issue arising with age is the increasing prevalence and severity of some infectious diseases, which partly reflects the age-related decline of immune function. Pneumonia, infections of the urinary tract and of the skin, as well as reactivation of latent pathogens, like the varicella zoster virus and *Mycobacterium tuberculosis,* are common in the elderly. Influenza, e.g,. is often associated with severe complications and secondary infections in the elderly. During an epidemic influenza season, three to five million cases of severe disease and 250,000 to 500,000 deaths occur worldwide [2]. In industrialized countries, most deaths associated with influenza occur among the elderly. In developed countries, like the United States, deaths form pneumonia and influenza account for more than 3% of all deaths in people aged 65 years and older [3]. Vaccination is of crucial importance in order to prevent infection and to protect the vulnerable elderly population from disease. As the efficacy of a vaccine depends on the quality of the immune response, immunocompromised persons, such as very young infants and elderly individuals, are likely to be insufficiently protected [4]. Thus, over the last decade, a large number of studies have shown that a variety of vaccines are less efficient in the elderly. Annual vaccination against influenza is, for instance, recommended in most developed countries for individuals with underlying chronic diseases, and for everybody above the age of 60 or 65, depending on individual national recommendations. However,

antibody responses after vaccination are lower in the elderly compared to young adults [5]. Especially in very old and frail persons decreased IgA and IgG antibody concentrations, delayed peak antibody titers and a faster decline of titers occur. For instance, seroprotection to influenza virus strains is only about 29-46% in persons aged ≥ 75 years compared to 41-58% in persons between 60 and 75 years of age (Table 1). Since non-adjuvanted influenza subunit vaccines show lower seroprotection and seroconversion rates compared to adjuvanted subunit, virosomal or split vaccines [6, 7], they should not be applied in elderly persons. There are more than 90 serotypes of *Streptococcus pneumoniae*, which frequently affect young children and elderly persons. 15-30% of pneumonia cases are associated with invasive pneumococcal disease (e.g. bacteremia or meningitis), with a case fatality rate of up to 40% for persons aged 85 years or older [8]. Currently, the 23-valent pneumococcal polysaccharide vaccine offers protection against invasive pneumococcal disease in the general elderly population (50-70%), but has only moderate effects in the high-risk elderly (20%) [9]. Moreover, the vaccine has only little effect against pneumonia. Although 7-valent conjugate polysaccharide vaccines have been developed that improve vaccine responses in young children, these vaccines failed do improve immunogenicity in the elderly [10].

Herpes zoster, which is caused by reactivation of the Varicella zoster virus, is another disease that predominantly occurs in the elderly. A live-attenuated virus vaccine aiming to prevent herpes zoster and postherpetic neuralgia has recently been introduced. Although vaccine efficacy is about 64% in the elderly population, only 18% of persons older than 80 years are protected [11]. Due to an increased travel activity of elderly persons, travel vaccinations become an increasingly important issue. For instance, vaccination against Hepatitis A induces protective antibody responses in only 63% of elderly vaccinees compared to 92% of young adults [12]. Another travel

vaccine aims to protect against yellow fever, which is endemic in tropic regions of Africa and South America. Due to the recent increase in the use of yellow fever vaccine in elderly persons, advanced age has been suggested to be a risk factor for adverse effects [13]. Moreover, booster vaccinations against tetanus, tick-borne encephalitis, pertussis and diphtheria induce a decreased response and have a shortened duration of protection in healthy elderly persons (Table 1) [14, 15].

The immune response following vaccination

Vaccines induce both innate (non-specific) and adaptive (specific) immune responses. Figure 1 schematically depicts the immune responses induced by vaccination and indicates possible age-related alterations. Protein antigens are usually injected together with adjuvants such as aluminum salts. Adjuvants retain antigen at the site of injection and/or stimulate local innate immune responses, such as the production of pro-inflammatory cytokines by macrophages [16]. This provides a “danger” signal [17] which supports the maturation of dendritic cells (DC). The antigen is taken up by macrophages or dendritic cells. DC are then activated and migrate to regional lymph nodes, where they present the processed antigen on their surface together with major histocompatibility (MHC) molecules. Inactivated vaccines are presented in the context of MHC class II molecules to $CD4^+$ T lymphocytes. Vaccination with live vaccines can lead to the intracellular production of antigenic peptides within antigen-presenting cells (APC), which are then presented to $CD8^+$ T lymphocytes in the context of MHC class I molecules. T cells recognize the MHC/antigen complex with their specific T cell receptors. This leads to T cell activation, clonal expansion of effector T cells and the formation of long-lived memory T cells, the hallmark of adaptive immunity. In the case of primary exposure to an antigen naïve, antigen-inexperienced T cells are activated. Upon booster vaccination, pre-existing memory T cells recognize antigen-loaded DC, expand rapidly and differentiate into effector T cells, which leads to a faster and

stronger memory response. $CD4^+$ T helper cells stimulate B cells which have been activated by contact with their specific antigens to differentiate into memory B cells and antibody-secreting B cells, which migrate to the periphery. These B cells have undergone recombination events that facilitate the expression of IgG instead of IgM antibodies, the so-called heavy chain isotype switching. Some of these B cells further differentiate into long-lived plasma cells that reside in the bone marrow. Antibodies circulate in the blood and enter the mucosa where they directly bind pathogens preventing entry into host cells and enhancing recognition by phagocytes. Like memory T cells, memory B cells are also capable of mounting fast and strong responses to secondary vaccination.

Antigens that have highly repetitive structures, such as bacterial polysaccharides are capable of inducing antibody responses without the need for T cell help [18]. Mature B cells directly bind repetitive elements of these antigens, which are either cell bound or soluble, via membrane-bound antibodies. Antigen-induced activation leads to differentiation into antibody-producing B cells which migrate to the periphery without the need for T cell help. Generally, T cell independent antibody responses are only short-lasting as differentiation into long-lived plasma cells is not induced. They have low affinity due to reduced antibody class switching to IgG and do not induce immunological memory. However, some T cell independent antigens, like the polysaccharide vaccine against *Streptococcus pneumoniae,* are believed to induce long-lived protective immunity. The underlying mechanisms are unclear. It has been speculated that antigens might persist for long periods in lymphoid tissue in cells such as follicular dendritic cells, where they continuously stimulate B cells.

Age-related changes of the immune system and their impact on vaccination

A declining immune function with age substantially contributes to the decreased efficacy of vaccines in the elderly. The underlying complex changes of the immune system are collectively termed immunosenescence; and they affect cell types of both the innate and the adaptive immune system.

The innate immune response in old age

Neutrophils and macrophages have a reduced phagocytic capacity and their oxidative burst is decreased in the elderly [19]. Additionally, the upregulation of MHC class II expression is impaired in old macrophages {van Duin, 2007 1097 /id}. Phagocytic cells recognize common structures of pathogens via Toll-like receptors (TLR). TLR-signaling leads to the efficient activation of the phagocytes and induces innate immune responses. Defects in the expression of TLR have been shown in macrophages of elderly persons [21]. The number of Langerhans cells in the skin also decreases with age and the expression of MHC class I and II, as well as the capacity to present antigen are reduced in DC from old mice [22]. All these age-related impairments of the innate immune response can hamper the success of vaccination by decreased uptake of antigen at the site of injection due to reduced phagocytosis. Defects in the processing and presentation of antigens lead to diminished activation and stimulation of adaptive immune cells.

In spite of functional defects of innate immune cells on a per cell basis, inflammatory processes occur ubiquitously with increasing age. This characteristic subclinical pro-inflammatory status has been termed “inflammaging”[23] and is known to be a predisposing factor for age related-diseases. Pro-inflammatory cytokines such as IL-6, IL-1β and TNF-α are produced at elevated levels in the elderly e.g. in the brain, in blood vessels and in bones. “Inflammaging” is believed to be due to chronic

stimulation of innate immunity by products of degradation processes and/or by the partial inability of the aged immune system to eliminate some pathogens, which may lead to chronic, yet inefficient innate immune responses.

These persistent inflammatory processes may hamper the aged organism's capacity to recognize stimuli induced by pathogens/vaccines as "danger" signals. Signals of higher intensity than operative in the young may be needed to induce DC maturation and adaptive immunity. Conventional vaccines and adjuvants may fail to reach this critical threshold at the site of injection in the elderly. Improved antigen-delivery systems (e.g. liposomes and virosomes), immunostimulatory adjuvants, such as saponins, adjuvants targeting Toll-like receptors (e.g. 3-deacetylated monophosphoryl lipid A (MPL) and CpG-oligodeoxynucleotides) and nanoparticles [24], or the administration of recombinant cytokines, might help to overcome these limitations. Presently, only two influenza vaccines containing new adjuvants are registered in Europe. One vaccine contains a virosomal formulation of influenza antigens, the other one uses an oil-in-water emulsion of saponin and other components (MF59®).

The adaptive immune response in old age

One of the most prominent events during aging is the continuous loss of thymic cortex and medulla, which starts already very early in life [25] (Figure 2). As a consequence, the output of mature naïve T cells from the thymus decreases with age leading to severely reduced numbers of naïve T cells in the periphery in the elderly [26, 27]. Naïve T cells are generally characterized by surface markers such as CD28, CD27, CD45RA and CCR7, by long telomeres, which indicates a short replicative history, and by a highly diverse T cell receptor (TCR) repertoire. In addition to the reduced number of phenotypically naïve ($CD45RA^+CD28^+$) T cells the remaining $CD45RA^+CD28^+$ T

cells are functionally deficient in elderly persons. They have shortened telomeres and a restricted TCR repertoire suggesting past homeostatic proliferation leading to the expansion of T cells with certain specificities and to the loss of others [28]. This numerical and functional impairment of naïve T cells hampers the induction of adaptive immune responses to neo-antigens. In the context of primary vaccination this leads to reduced response rates. Naïve antigen-inexperienced T cells are also functionally deficient and difficult to prime in aged mice [29].

Memory T cells are crucial in controlling humoral and cellular immune responses. For sustained protective immunity it is therefore necessary to induce a functional T cell memory following immunizations. Experiments in mice have shown that memory T cells generated from aged naïve T cells survive and persist well *in vivo* but they are markedly defective in their proliferation and cytokine secretion during recall responses. In contrast, memory cells generated in young animals retain their function for extended periods of time [29]. Similar to aged mice, healthy elderly individuals are able to mount a T cell response after vaccination, but exhibit an impaired long-term immune response [30]. These findings emphasize the importance of early primary immunization in order to guarantee intact immunological memory in old age.

The decline in naïve T cell numbers in elderly persons is accompanied by the accumulation of highly differentiated effector T cells. Characteristics of highly differentiated effector T cells are short telomers, a highly restricted T cell receptor repertoire, an impaired capacity to migrate to lymph nodes and to be stimulated by antigen presenting cells due to the loss of the co-stimulatory molecules CD28 and CD27 [31]. $CD28^-$ effector T cells also produce high amounts of the pro-inflammatory cytokine interferon-γ, which contributes to the high inflammatory background typical of old age [32], supporting the development and progression of age-related diseases such as osteoporosis, atherosclerosis and neurodegeneration [33]. The accumulation

of $CD28^-$ effector T cells has also been shown to be correlated with impaired humoral responses to influenza vaccination [34, 35]. However, $CD28^-$ effector T cells are a heterogeneous cell population, in particular in old age, and some studies suggest that a subpopulation of these cells has functional defects such as for instance, increased expression of PD1 [36] Interestingly, chronic infections substantially contribute to the replicative exhaustion of the peripheral T cell pool. Life-long infection with cytomegalovirus (CMV) is associated with increased numbers of CMV-specific $CD28^-$ effector T cells in young and elderly persons [37]. These cells occur as expanded clones and dominate the peripheral T cell pool, hampering the propagation of other T cell specificities (e.g. Epstein-Barr virus-specific T cells) [38] and thus endangering diversity [39], homeostasis and successful immunization within the aged immune system.

B cells also undergo age-related changes, which further aggravate functional defects of the adaptive immune response. Similar to the T cell system, naïve B cell numbers decrease and effector B cells accumulate in old age. This leads to a reduction in the diversity of antibody responses [40]. Defects in isotype-switching and somatic mutation, both of which are essential for the production of high-affinity IgG antibodies result in weak and low-affinity antibody responses in the elderly [41]. The number of long-lived plasma cells in the bone-marrow is reduced in aged mice probably due to an impaired ability of the aged bone marrow to support survival of plasma cells [42]. Additionally, interactions of aged B cells with T helper cells are disturbed in the elderly as senescent $CD4^+$ T helper cells have a reduced expression of CD154 (CD40L) [43], a molecule of crucial importance for the stimulation of B cells by T cells.

As antibody titers are declining faster in the elderly [14] and as the success of booster vaccination clearly correlates with pre-booster antibody titers [15], optimization of

immunization schedules seems advisable. Regular vaccination during young adulthood may be a prerequisite for successful booster vaccination in old age.

Little information is still available on the effect of live-attenuated vaccines on the aged immune system. Childhood vaccination with these vaccines should per definition induce long-lasting antibody production and strong cytotoxic T lymphocyte responses. However, as routine vaccinations of children against measles, mumps and rubella have only been performed for less than 40 years, there is no data available whether childhood immunization with live-attenuated vaccines is still protective in old age. A recent publication reports that the number of $CD4^+$ T cells specific for measles declines with age in vaccinated individuals [44]. It will be important to determine whether booster immunizations would be advisable for persons vaccinated with live vaccines during childhood. Primary immunization with live-attenuated vaccines in old age may be associated with an increased risk of side effects. Thus, it has been suggested that advanced age is a risk factor for severe systemic side effects associated with the application of yellow fever vaccine [13]. This may be due to low numbers and functional defects of naïve T cells indicating that an aged organism may not be capable to cope with a new pathogen even in the case of attenuated vaccine-strains. In order to avoid side-effects in the elderly, safer live-vaccines are needed. The first generation of live-vaccines was produced by serial passages in cell culture, which leads to an unspecific loss of pathogenicity. Development of new live-vaccines includes rationally designed attenuation, e.g. by the deletion of individual genes. These new vaccines should be specifically tested for the use in elderly persons and should provide an improved safety profile.

An alternative strategy for successful immunization with live-vaccines might be to apply live-attenuated vaccines only earlier in life followed by booster vaccination with inactivated or adjuvanted subunit vaccines in old age.

Conclusion

The frequency and severity of infectious diseases increase with old age. Infections harbor a substantial risk of illness, loss of independence, disability and death in elderly persons. They, thereby, contribute to the socio-economic burden associated with rising life expectancy. Vaccinations provide efficient protection from infectious diseases. Age-related changes in the immune system may hamper successful vaccination. Vaccines tailored for the needs of the aging immune system will have to be developed and vaccination schedules will have to be adapted to improve protection in the elderly. A better insight into the basic mechanisms of immune dysfunctions that occur with age will help to fulfill this task in order to ensure protection of the vulnerable elderly population.

Acknowledgement

This work was supported by the Austrian Science Fund (Project S9308-B05) and the EU (Project EUP 0134 “LifeSpan” (Integrating research into development and ageing)).

B.W., D. H.-B., A.S., D.W. and B. G.-L. declare no conflict of interest.

References

1. Lutz, W, Sanderson, W, Scherbov, S. Doubling of world population unlikely. Nature. **1997**; 19:803-805.

2. World Health Organiszation. Influenza fact sheet No. 211. Available at: www.euro.who.int. Accessed 24 July 2007.

3. Heron, MP and Smith, BL. Deaths: leading causes for 2003. Natl.Vital Stat.Rep. **2007**; 55:1-92.

4. Grubeck-Loebenstein, B and Wick, G. The aging of the immune system. Adv.Immunol. **2002**; 80:243-284.

5. Goodwin, K, Viboud, C, Simonsen, L. Antibody response to influenza vaccination in the elderly: a quantitative review. Vaccine. **2006**; 24:1159-1169.

6. Conne, P, Gauthey, L, Vernet, P et al. Immunogenicity of trivalent subunit versus virosome-formulated influenza vaccines in geriatric patients. Vaccine. **1997**; 15:1675-1679.

7. De Donato, S, Granoff, D, Minutello, M et al. Safety and immunogenicity of MF59-adjuvanted influenza vaccine in the elderly. Vaccine. **1999**; 17:3094-3101.

8. Artz, AS, Ershler, WB, Longo, DL. Pneumococcal vaccination and revaccination of older adults. Clin.Microbiol.Rev. **2003**; 16:308-318.

9. Melegaro, A and Edmunds, WJ. The 23-valent pneumococcal polysaccharide vaccine. Part I. Efficacy of PPV in the elderly: a comparison of meta-analyses. Eur.J.Epidemiol. **2004**; 19:353-363.

10. Powers, DC, Anderson, EL, Lottenbach, K, Mink, CM. Reactogenicity and immunogenicity of a protein-conjugated pneumococcal oligosaccharide vaccine in older adults. J.Infect.Dis. **1996**; 173:1014-1018.

11. Oxman, MN, Levin, MJ, Johnson, GR et al. A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. N.Engl.J.Med. **2005**; 352:2271-2284.

12. Wolters, B, Junge, U, Dziuba, S, Roggendorf, M. Immunogenicity of combined hepatitis A and B vaccine in elderly persons. Vaccine. **2003**; 21:3623-3628.

13. Martin, M, Weld, LH, Tsai, TF et al. Advanced age a risk factor for illness temporally associated with yellow fever vaccination. Emerg.Infect.Dis. **2001**; 7:945-951.

14. Hainz, U, Jenewein, B, Asch, E, Pfeiffer, KP, Berger, P, Grubeck-Loebenstein, B. Insufficient protection for healthy elderly adults by tetanus and TBE vaccines. Vaccine **2005**; 23:3232-3235.

15. Kaml, M, Weiskirchner, I, Keller, M et al. Booster vaccination in the elderly: Their success depends on the vaccine type applied earlier in life as well as on pre-vaccination antibody titers. Vaccine. **2006**; 24:6808-6811.

16. HogenEsch, H. Mechanisms of stimulation of the immune response by aluminum adjuvants. Vaccine. **2002**; 20 Suppl 3:S34-9.:S34-S39.

17. Matzinger, P. The danger model: a renewed sense of self. Science. **2002**; 296:301-305.

18. Mond, JJ, Lees, A, Snapper, CM. T cell-independent antigens type 2. Annu.Rev.Immunol. **1995**; 13:655-92.:655-692.

19. Gomez, CR, Boehmer, ED, Kovacs, EJ. The aging innate immune system. Curr Opin Immunol **2005**; 17:457-62.

20. van, DD and Shaw, AC. Toll-like receptors in older adults. J.Am.Geriatr.Soc. **2007**; 55:1438-1444.

21. Renshaw, M, Rockwell, J, Engleman, C, Gewirtz, A, Katz, J, Sambhara, S. Cutting edge: impaired Toll-like receptor expression and function in aging. J.Immunol. **2002**; 169:4697-4701.

22. Grewe, M. Chronological ageing and photoageing of dendritic cells. Clin.Exp.Dermatol. **2001**; 26:608-612.

23. Franceschi, C, Olivieri, F, Marchegiani, F et al. Genes involved in immune response/inflammation, IGF1/insulin pathway and response to oxidative stress play a major role in the genetics of human longevity: the lesson of centenarians. Mech.Ageing Dev. **2005**; 126:351-361.

24. Reddy, ST, van, d, V, Simeoni, E et al. Exploiting lymphatic transport and complement activation in nanoparticle vaccines. Nat.Biotechnol. **2007**; 25:1159-1164.

25. Steinmann, GG, Klaus, B, Muller-Hermelink, HK. The involution of the ageing human thymic epithelium is independent of puberty. A morphometric study. Scand.J.Immunol. **1985**; 22:563-575.

26. Fagnoni, FF, Vescovini, R, Passeri, G et al. Shortage of circulating naive CD8(+) T cells provides new insights on immunodeficiency in aging. Blood **2000**; 95:2860-8.

27. Lazuardi, L, Jenewein, B, Wolf, AM, Pfister, G, Tzankov, A, Grubeck-Loebenstein, B. Age-related loss of naive T cells and dysregulation of T-cell/B-cell interactions in human lymph nodes. Immunology **2005**; 114:37-43.

28. Pfister, G, Weiskopf, D, Lazuardi, L et al. Naive T cells in the elderly: are they still there? Ann.N.Y.Acad.Sci. **2006**; 1067:152-7.:152-157.

29. Haynes, L. How vaccines work on the background of the aging immune system. Exp.Gerontol. **2007**; 42:438-440.

30. Kang, I, Hong, MS, Nolasco, H et al. Age-associated change in the frequency of memory CD4+ T cells impairs long term CD4+ T cell responses to influenza vaccine. J.Immunol. **2004**; 173:673-681.

31. Appay, V, Dunbar, PR, Callan, M et al. Memory CD8+ T cells vary in differentiation phenotype in different persistent virus infections. Nat.Med. **2002**; 8:379-385.

32. Almanzar, G, Schwaiger, S, Jenewein, B et al. IFN-gamma production by CMV-specific CD8+ T cells is high in elderly donors. Exp.Gerontol. **2004**; 39:863-865.

33. Blasko, I, Stampfer-Kountchev, M, Robatscher, P, Veerhuis, R, Eikelenboom, P, Grubeck-Loebenstein, B. How chronic inflammation can affect the brain and support the development of Alzheimer's disease in old age: the role of microglia and astrocytes. Aging Cell **2004**; 3:169-176.

34. Saurwein-Teissl, M, Lung, TL, Marx, F et al. Lack of antibody production following immunization in old age: association with CD8(+)CD28(-) T cell clonal expansions and an imbalance in the production of Th1 and Th2 cytokines. J.Immunol. **2002**; 168:5893-5899.

35. Goronzy, JJ, Fulbright, JW, Crowson, CS, Poland, GA, O'Fallon, WM, Weyand, CM. Value of immunological markers in predicting responsiveness to influenza vaccination in elderly individuals. J.Virol. **2001**; 75:12182-12187.

36. Day, CL, Kaufmann, DE, Kiepiela, P et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature. **2006**; 443:350-354.

37. Almanzar, G, Schwaiger, S, Jenewein, B et al. Long-term cytomegalovirus infection leads to significant changes in the composition of the CD8+ T-cell repertoire, which may be the basis for an imbalance in the cytokine production profile in elderly persons. J.Virol. **2005**; 79:3675-3683.

38. Khan, N, Hislop, A, Gudgeon, N et al. Herpesvirus-specific CD8 T cell immunity in old age: cytomegalovirus impairs the response to a coresident EBV infection. J Immunol **2004**; 173:7481-9.

39. Hadrup, SR, Strindhall, J, Kollgaard, T et al. Longitudinal studies of clonally expanded CD8 T cells reveal a repertoire shrinkage predicting mortality and an increased number of dysfunctional cytomegalovirus-specific T cells in the very elderly. J.Immunol. **2006**; 176:2645-2653.

40. Allman, D and Miller, JP. B cell development and receptor diversity during aging. Curr Opin Immunol **2005**; 17:463-7.

41. Frasca, D, Riley, RL, Blomberg, BB. Humoral immune response and B-cell functions including immunoglobulin class switch are downregulated in aged mice and humans. Semin.Immunol. **2005**; 17:378-384.

42. Han, S, Yang, K, Ozen, Z et al. Enhanced differentiation of splenic plasma cells but diminished long-lived high-affinity bone marrow plasma cells in aged mice. J.Immunol. **2003**; 170:1267-1273.

43. Haynes, L and Eaton, SM. The effect of age on the cognate function of CD4+ T cells. Immunol.Rev. **2005**; 205:220-8.:220-228.

44. Naniche, D, Garenne, M, Rae, C et al. Decrease in measles virus-specific CD4 T cell memory in vaccinated subjects. J.Infect.Dis. **2004**; 190:1387-1395.

45. Di Rosa A. and Pabst, R. The bone marrow: a nest for migratory memory T cells. Trends Immunol. **2005**; 26:360-366.

46. D'Acremont, V, Herzog, C, Genton, B. Immunogenicity and safety of a virosomal hepatitis A vaccine (Epaxal) in the elderly. J.Travel.Med. **2006**; 13:78-83.

47. Khromava, AY, Eidex, RB, Weld, LH et al. Yellow fever vaccine: an updated assessment of advanced age as a risk factor for serious adverse events. Vaccine. **2005**; 23:3256-3263.

48. Monath, TP, Cetron, MS, McCarthy, K et al. Yellow fever 17D vaccine safety and immunogenicity in the elderly. Hum.Vaccin. **2005**; 1:207-214.

Figure legends

Figure 1 Schematic representation of the immune responses and its age-related alterations following vaccination.

Protein antigens administered together with adjuvants induce the activations of innate immune responses at the site of injection. The antigen is taken up by antigen presenting cells (1), such as macrophages and dendritic cells (DC). The local innate immune response facilitates maturation of DC, which present stable peptide-MHC complexes (2). Mature DC migrate into lymph nodes (3) where they induce activation and clonal expansion of naïve $CD4^+$ (4) and $CD8^+$ (5) T cells. The activation and differentiation of naïve B cells is induced by antigen and $CD4^+$ T cell help (6). Naïve B cells differentiate into memory B cells and antibody secreting B cells (7). Long-term immunity is assured by memory B and T cells in the blood and lymph nodes as well as by long-lived plasma cells and memory T cells in the bone marrow [45].

Figure 2 Schematic representation of the age-dependent involution of functional thymic tissue.

The volume of functional thymic tissue (cortex and medulla) progressively declines during aging and is replaced by adipose tissue. Data from [25].

Table 1

Disease	Vaccine type	Vaccine efficacy in elderly persons	Ref.
Influenza	Inactivated virus, subunit, adjuvanted subunit and virosome	A / H1N1: 55% (32%) [a] A / H3N2: 58% (46%) B: 41% (29%)	[5]
Hepatitis A	Inactivated virus Virosome	63% [b] 65% (97%) [c]	[12] [46]
Hepatitis B	Subunit	33% [b]	[12]
Herpes Zoster	Live-attenuated virus	64 (18%) [d]	[11]
Pertussis	Toxoid and acellular components	> 81% [e]	[15]
Pneumonia	Non-conjugated polysaccharide	50-70%	[8]
Poliomyelitis	Inactivated virus	99% [f]	[15]
Tetanus and Diphtheria	Toxoid	99% and 84% [g]	[15]
Tick-borne encephalitis	Inactivated virus	70% [h]	[14]
Yellow fever	Live-attenuated virus	100%	[47, 48]

[a] Seroprotection (%) of persons with a mean age between 65 and 75 years (and a mean age ≥ 75 years) from three different influenza virus strains
[b] Seroprotection (anti-HAV antibody concentration ≥ 20 IU/L; anti-HBs antibody concentration of at least 10 IU/L) of persons ≥ 60 years after two booster vaccinations
[c] Seroprotection (≥ 20 IU/L) of persons ≥ 50 years of age after primary (and booster) vaccination
[d] Vaccine efficacy in 60-69 year old and in (≥ 80 year old) persons
[e] Percentage of elderly persons (median age: 66 years) with protective antibody levels against pertussis after vaccination with Repevax®
[f] Vaccine efficacy in elderly persons (median age: 66 years) after booster vaccination with Repevax®
[g] Percentage of elderly persons (median age: 66 years) with protective antibody levels against Tetanus and Diphtheria after booster vaccination with Repevax®
[h] Percentage of elderly persons (> 60 years) with protective antibody levels (≥ 100 VIE.U./mL) against TBE after booster vaccination 3-4 years after the last vaccination

Figure 1

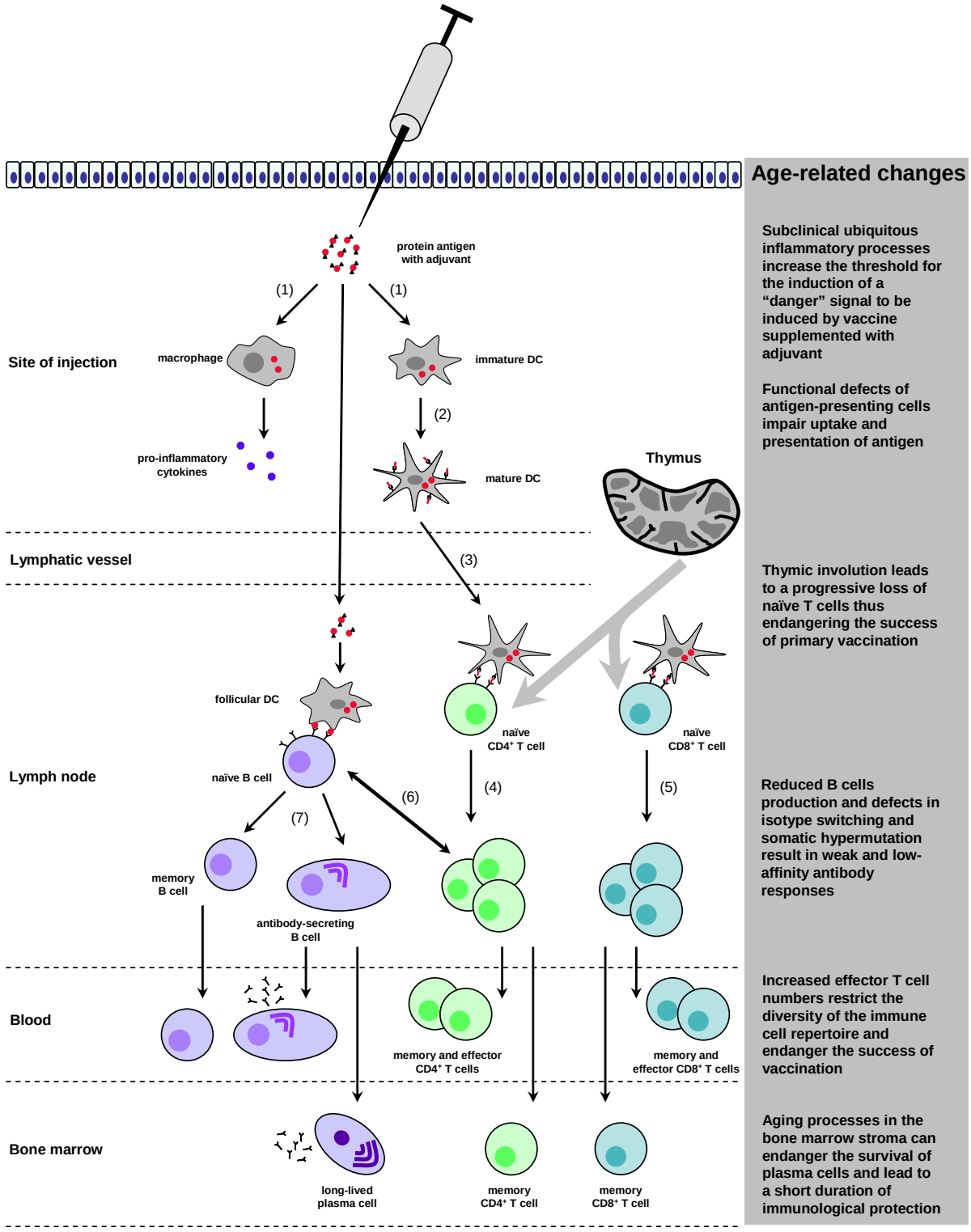
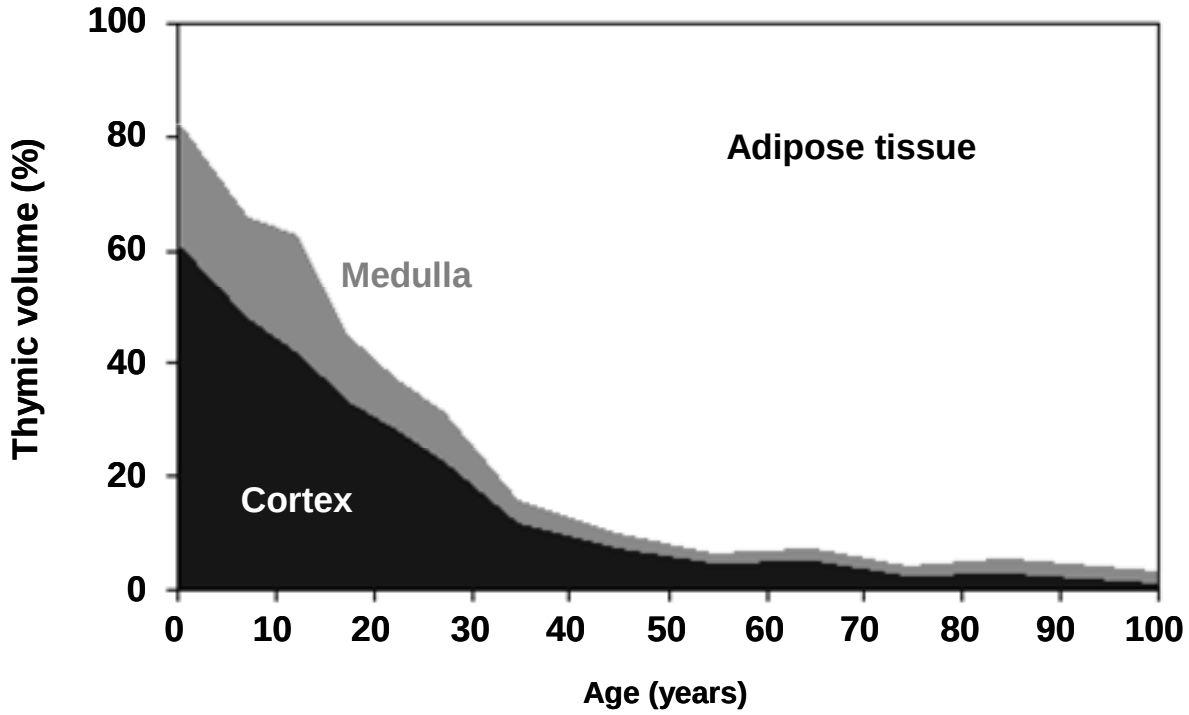

protein antigen with adjuvant
(1)
(1)
Site of injection
macrophage
immature DC
(2)
pro-inflammatory cytokines
mature DC
Thymus
Lymphatic vessel
(3)
follicular DC
naïve CD4⁺ T cell
naïve CD8⁺ T cell
Lymph node
naïve B cell
(6)
(4)
(5)
(7)
memory B cell
antibody-secreting B cell
Blood
memory and effector CD4⁺ T cells
memory and effector CD8⁺ T cells
Bone marrow
long-lived plasma cell
memory CD4⁺ T cell
memory CD8⁺ T cell
Age-related changes
Subclinical ubiquitous inflammatory processes increase the threshold for the induction of a “danger” signal to be induced by vaccine supplemented with adjuvant
Functional defects of antigen-presenting cells impair uptake and presentation of antigen
Thymic involution leads to a progressive loss of naïve T cells thus endangering the success of primary vaccination
Reduced B cells production and defects in isotype switching and somatic hypermutation result in weak and low-affinity antibody responses
Increased effector T cell numbers restrict the diversity of the immune cell repertoire and endanger the success of vaccination
Aging processes in the bone marrow stroma can endanger the survival of plasma cells and lead to a short duration of immunological protection

Figure 2


Thymic volume (%)
100
80
60
40
20
0
Adipose tissue
Medulla
Cortex
0
10
20
30
40
50
60
70
80
90
100
Age (years)