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Vaccination in the elderly: The challenge of immune changes with aging

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ABSTRACT

The unprecedented increase of life expectancy challenges society to protect the elderly from morbidity and mortality making vaccination a crucial mean to safeguard this population. Indeed, infectious diseases, such as influenza and pneumonia, are among the top killers of elderly people in the world. Elderly individuals are more prone to severe infections and less responsive to vaccination prevention, due to immunosenescence combined with the progressive increase of a proinflammatory status characteristic of the aging process (inflammaging). These factors are responsible for most age-related diseases and correlate with poor response to vaccination. Therefore, it is of utmost interest to deepen the knowledge regarding the role of inflammaging in vaccination responsiveness to support the development of effective vaccination strategies designed for elderly.

In this review we analyse the impact of age-associated factors such as inflammaging, immunosenescence and immunobiography on immune response to vaccination in the elderly, and we consider systems biology approaches as a mean for integrating a multitude of data in order to rationally design vaccination approaches specifically tailored for the elderly.

1. Need for vaccines designed for the elderly

The demographic revolution occurred in the last 100 years has led to a consistent increase of life expectancy and a proportional growth of the elderly population. The population in high income countries is aging rapidly, and between 2015 and 2050, the proportion of the world's population over 60 years is expected to nearly double from 12% to 22% [1].

Unfortunately, the average prolongation of lifespan is not fully paralleled by a prolongation of the health-span. This demographic dynamic is posing critical burdens for the majority of the health care systems, stretched by the rising costs of care of this ever-growing fragile portion of the population. Preventive medicine is the most effective and feasible strategy to protect health in old subjects and vaccination against the most common infectious diseases is the most indicated approach. Seasonal influenza, pneumococcus infection and reactivation of varicella zoster virus are three harmful pathological conditions that represent causes of significant morbidity and mortality for old people, more susceptible than young adults.

The vaccination recommendations for the elderly established in the USA and in Europe include as main target diseases the aforementioned seasonal influenza, pneumococcal disease and reactivation of varicella zoster virus (VZV), with vaccination schedules differing from country to country (Table 1). Regular booster shots against tetanus, diphtheria, pertussis, polio are also recommended in the elderly, and in some countries (Austria, France, Liechtenstein and Portugal) the booster intervals are shortened for persons over 65 years as the result of a more rapid decline in antibodies with advancing age [2]. Nevertheless, while compliance with vaccine recommendations in children is generally high, reaching over 90% coverage in most high-income countries, it is far lower in adults [3] and the burden of vaccine-preventable diseases in terms of morbidity, mortality and direct and indirect costs remains high.

Most currently used vaccines are less immunogenic and effective in the elderly compared to younger adults [4]. This is due to several factors, including the fact that most of the vaccines are specifically designed for children and young adults with an immune system that is different from

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Table 1
Influenza, pneumococcal and herpes zoster vaccine schedules recommended for elderly in countries of the European Union and in USA.

		Influenza[a]	Pneumococcal disease	Herpes zoster
Europe				
	Austria	> 65	> 50	> 50
	Belgium	> 65	> 86	
	Bulgaria	> 65	> 2 months (mandatory)	
	Croatia	> 65		
	Cyprus	> 65	> 65	
	Czech Republic	> 65	> 65	> 50
	Denmark	> 65	> 65	
	Estonia	> 65		
	Finland	> 65	> 65	
	France	> 65	> 2 months (mandatory)	> 65
	Germany	> 60	> 50	
	Greece	> 60	> 65	> 60
	Hungary	> 60	> 2 months (mandatory); > 50	
	Iceland	> 60	> 50	
	Ireland	> 65	> 65	
	Italy	> 65	> 65	> 65
	Latvia	> 65	> 2 months (mandatory)	
	Liechtenstein	> 65		
	Lithuania	> 65		
	Luxembourg	> 65	> 65	
	Malta	> 55	> 65	
	Netherlands	> 65		
	Norway	> 65	> 65	
	Poland	> 55	> 2 months (mandatory); > 50	
	Portugal	> 65	> 65	
	Romania	> 65	> 65	
	Slovakia	> 60	> 2 months (mandatory); > 60	
	Slovenia	> 18	> 65	
	Spain	> 65	> 65	
	Sweden	> 65	> 65	
	United Kingdom	> 65	> 65	> 70
USA		> 18	> 65	> 50

[a] Most countries recommend IIV.

elderly people, where physiological immunosenescence coexists with the personal history of infections and vaccinations. As an example, the ability of influenza vaccine to induce protection is related to age, with an efficacy between 70% and 90% in children and adults, but dropping to 30–50% for those over 65 years of age [4,5]. Similarly, responses to pneumococcal polysaccharide and hepatitis B vaccines are compromised by old age, and antibody responses are of shorter duration in older people [6].

The value of vaccines for the elderly relies not only on efficacy, an indicator of the vaccine ability to confer protection against a specific infection, but also on effectiveness, that is a measure of the capacity of generally improving the health status of the older individual avoiding other related diseases. It is therefore of primary importance to design vaccination strategies specifically tailored on the elderly population, both in terms of vaccine formulations and vaccination protocols, taking in consideration the aging immune system and inflammaging, two essential characteristics of aging, described more in detail in Section 2. This priority has also been highlighted in the recent European roadmap for vaccine development [7].

A deeper understanding of optimal strategies to stimulate the elderly immune system would have an enormous impact not only in the optimization of existing vaccines but also in guiding the development of novel vaccines highly needed for the elderly such as those against respiratory syncytial virus (RSV), antibiotic-resistant bacteria more frequent in this age group also due to hospitalization (including *Staphylococcus aureus, Clostridium difficile, Candida* spp., *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae and Acinetobacter baumannii*), as well as in the design of therapeutic cancer vaccines [8].

To allow these goals to be in closer reach, we first review the current knowledge on the elderly immune system and response to vaccination and we propose to focus on the relevance of vaccine adjuvants, on immunobiography, i.e. the lifelong exposures to antigenic stimuli leading to the individual immunological elderly phenotype [9], and on systems approaches to enable the future design of effective vaccines tailored on the elderly.

2. Immune system in aging

2.1. Immunosenescence

As a result of the immune function decline, elderly subjects do not respond efficiently to novel or previously encountered antigens. Indeed, with aging, the immune system of elderly is remodelled with fewer naïve cells and increase in dysfunctional memory cells, as well as primary lymphoid organs involution and altered innate immune response, leading to greater susceptibility to infectious diseases and reduced responses to vaccination (Fig. 1).

Nevertheless, we should consider the differences measured between elderly individuals and young adults as an adjustment over the life course to the requirements of the ecological environment where the individual evolves, rather than an abnormal process [10]. Overall, as a result of immunosenescence, the elderly population is more susceptible to infections, in particular to influenza, pneumococci, RSV and group B streptococcus but also to opportunistic and re-emergent chronic infections such as herpes zoster.

The complex biological processes of aging are the result of a network of events regarding different cell types and tissues, alterations in gene regulation and protein expression, signalling pathways and biological networks. Different cell populations such as neutrophils, monocytes and dendritic cells (DC) are altered, and their functions are reduced, including chemotaxis, phagocytosis, signalling pathways and intracellular killing via free radical production. Moreover, a set of phenomena that are better described by the concept of inflammaging, detailed in section 2.2, are relevant in this context.

Adaptive immunity undergoes profound and complex changes with age, including pervasive epigenetic and metabolic modifications, affecting most of the subsets of naïve, memory and effector T cells, T regulatory (T reg) and B cells [11–13]. Reduced amounts of naïve T and B cells, increased numbers of memory cells and shrinkage of T cells repertoire owing to large clonal expansion towards epitopes of persistent viral infections (cytomegalovirus [CMV] and Epstein Barr virus [EBV]), are some of the major changes associated with aging [14,15]. Immunosenescence involves decreased efficiency of the adaptive immune system, such as naïve B and T cells production rate as well as composition and quality of the mature lymphocyte pool. The effects of aging on the immune system are widespread and affect the development of naïve lymphocytes and their cellular profile. Primary lymphopoiesis in the elderly is significantly reduced, mainly due to changes in progenitor cells [16–18]. In bone marrow, hematopoietic stem cells (HSC) shifts have been shown from lymphoid-biased to myeloid-biased subsets, as demonstrated by the increased expression of myeloid lineage genes and downregulation of those specifying a lymphoid lineage fate [19]. It has been recently reported that HSC from young or aged mice regenerate distinct adaptive immune system upon transplantation into RAG1-/- mice that resemble the T and B cell systems of young and aged mice [20]. Therefore, changes in the function of HSCs are among the main responsible of both phenotypic and functional modification in the immune system of the elderly.

The bone marrow significantly decreases with age and allows fat deposits in the marrow cavity as a result of the differentiation of stromal cells to adipocyte-like cells [21]. This leads to a decrease in the absolute number of early B cell progenitors, including both pro-B and pre-B cells, shifting from a B cell compartment rich in naïve B cells but with few memory B cells to one with reversed proportions in older age [6,12]. Moreover, lymphoid-biased stem cells show a decline in lymphopoiesis, common lymphoid progenitors, pre–pro-B cells, and pro-B cells from old mice do not proliferate as extensively as young cells do, and they exhibit significantly higher rates of apoptosis [22].

In addition to the strong changes in the lymphocyte development

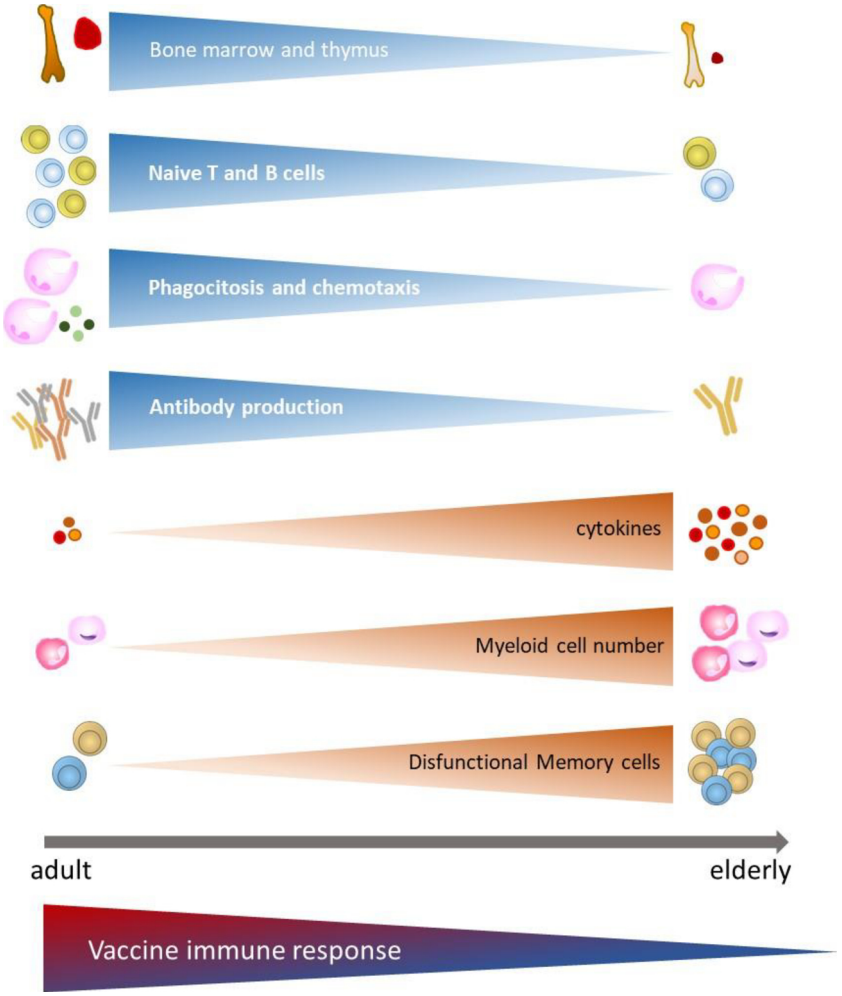

Fig. 1. Vaccine immune response in function of changes of the innate and adaptive immune system associated with aging. With aging, both the innate and the adaptive immune responses decrease, leading to reduced responses to vaccination. Immunosenescence involves the involution of primary lymphoid organs (bone marrow and thymus) with a reduction of B and T cells progenitors, dysfunctional memory cells, due to chronic antigenic stimulation (including, but not limited to, CMV), reduction of phagocyte functions (such as chemotaxis and phagocytosis), with concomitant increased levels of pro-inflammatory cytokine production. All these changes correlate with a decline in the immune response to vaccination.

compartment, the composition and the quality of the mature lymphocyte pool is also profoundly changed in the elderly. An increased pool of memory B cells has been observed, with limited repertoire diversity, that is not paralleled by a similar increase in the number of plasma cells [23]. Age-related autonomous B cell defects include a reduction in activation-induced cytidine deaminase (AID), the enzyme necessary for class switch recombination, somatic hypermutation, and IgG production, as well as in E47, the key transcription factor regulating AID [24,25]. Therefore, the percentage of switched memory B cells, the predictors of optimal antibody responses, decreases with age [25], while late memory B cells, the antigen-experienced and proinflammatory B cell subset, increase [26]. The latter is a highly inflammatory B cell subset, with characteristics of cell senescence such as reduced telomerase activity [27] and poor ability to proliferate *in vitro* in response to mitogenic stimulation, even though they are transcriptionally active, and express RNA for multiple senescence-associated secretory phenotype (SASP) markers, such as the pro-inflammatory cytokines TNF-α/IL-6/IL-8 and for the pro-inflammatory micro-RNAs (miRs)-155/16/93 [28]. Through secretion of these pro-inflammatory mediators, late memory B cells affect the microenvironment and in turn sustain and propagate the inflammatory response and negatively regulate the function of other immune cells. Another characteristic of the B cell responses in the elderly is their serological profile, that shows the increased presence of autoantibodies and low affinity antibodies and, in some subjects, the occurrence of an over-representation of specific classes of antibody from individual B cell clones [6]. Similarly, the thymus, the site of T cell development, is regressed in the elderly, with a consistent reduction of epithelial cells and increase of infiltrating adipocytes [29]. Mitogen-induced proliferation of T cells from older individuals shows that T cells have considerably less proliferative capacity *in vitro* compared with T cells from younger individuals [30]. This phenomenon is often coupled with the accumulations of late-stage memory CD8+ T cells, resulting from persistent infections such as CMV. This chronic viral infection can direct an oligoclonal expansion of memory cells, typically characterized in humans by the loss of the co-stimulatory molecule CD28 and impaired immune function, however the full implications of this infection with aging and immunosenescence are still matter of debate [31]. A gradual decline in functional response of memory and effector T cells is also held responsible of reduced response to vaccination in older persons [32,33].

Immunosenescence also compromises the ability of CD4+ T cells to differentiate into functional subsets resulting in a multitude of dysregulated responses, including a reduced cognate help to B cells, thus impacting humoral immunity [34]. Moreover the ratio of Th17 cells, a proinflammatory subset of CD4 + T cells, to T reg appears to increase in elderly people thus favouring a basal proinflammatory status [13,35].

These changes in the immune system of the elderly are the basis of the reduced response to vaccination and call in for the design of vaccination

strategies specifically tailored to optimally stimulate the aging immune system.

2.2. Inflammaging

Inflammaging is a major immunological characteristic of the elderly, defined as the progressive onset of a chronic, sterile and low-grade inflammation [36] recognized as a general etiological agent for age-related pathologies, extensively revised in [37–39]. Inflammaging strongly impacts on elderly subjects susceptibility to communicable disease and on the immune response to vaccination, therefore it should be taken in consideration for the design of vaccine formulations, including adjuvants. Several processes may contribute to inflammaging: a variety of organs and tissues damages due to increase of cells death rate and senescence, mitochondrial dysfunction, inflammasome and NFkB activation, circulating miRNAs, chronic infections such as CMV, hormones and reactive oxygen species -ROS [40], age related changes of nutrition, metabolism, α-galactosilated N-glycans [41] and host gut microbiota (GM) dysbiosis [42]. In particular, acute and chronic viral and bacterial infections and self-generated misplaced/altered/aggregated proteins and cell debris - largely resulting from the continuous death of cells occurring in the body - appear to be the major stimuli fueling inflammaging. Inflammaging is therefore particularly important for vaccinology, owing to recent data suggesting that increased level of inflammatory cytokines in immune cells (such as macrophages, NK-cells, DC, late memory B cells and Th17 lymphocytes) and in blood correlate with poor response to vaccines [43].

Recently it has been reported in mouse studies that the GM exerts its regulatory effects not only on intestinal immunity but also on systemic immune responses and systemic T cell subset populations whose distribution can be skewed by different microbiota predominance [44–46]. Age-related microbiota changes drive intestinal permeability, systemic inflammation, and macrophage dysfunction, thus promoting age-associated inflammation [47]. Influenza viral infection affects GM composition, at the same time the immune response to influenza infection is affected by age and GM [48].

In a study from Biagi et al. [49] the authors described for the first time the changes occurring in the human GM with age and extreme longevity and showed that a profound remodeling occurs progressively with age and correlates to inflammaging.

3. Currently recommended vaccines for the elderly

3.1. Influenza vaccine

Influenza infection is one of the main causes of morbidity and mortality in the elderly. Worldwide, influenza annual epidemics are estimated to result in about 3–5 million cases of severe illness, and about 290.000–650.000 deaths [50]. Annual influenza vaccination is considered the most effective strategy to prevent influenza by the World Health Organization (WHO) and it is recommended for the elderly people in numerous high-income countries. However, the efficacy of influenza vaccines is reduced to 30–50% in elderly subjects as compared to 70–90% in adults [4,5]. In the USA, adult influenza vaccination is recommended for persons aged > 19 years who do not have contraindications [51], while in Europe annual vaccination recommendations vary widely among Member States (Table 1) [52]. Two types of influenza vaccine are currently available: inactivated influenza vaccine (IIV) and live attenuated influenza vaccine (LAIV). The trivalent inactivated influenza vaccine (TIV) contains antigens from two subtypes of influenza A strain (H1N1 and H3N2) and from one strain of influenza B (Victoria or Yamagata lineages). The frequent observation of co-circulation of the two B lineages and the frequent mismatch between the vaccine component and the circulating strains have prompted vaccine manufacturers to produce quadrivalent inactivated influenza vaccines (QIV) containing the two A strains and two B strains [53] that have now been licensed in some countries. LAIV, first licensed and used in Russia and in North America in 2003, is mainly recommended in children. Influenza viruses are constantly changing, due to the so-called “antigenic drift”, which consists of the spontaneous modification of the surface proteins hemagglutinin (HA) and neuraminidase (NA). For this reason, the vaccine composition has to be adapted annually to integrate viral strains as similar as possible to the epidemic strains. The amount of antigen of an influenza-inactivated vaccine is calculated on the content of HA, that is not less than 15 μg of each of the three (or four) HAs. The second component is NA; various amounts of internal proteins, like M1 and NP, can also be detected [54].

The effectiveness of TIV in the elderly is modest, ranging from 30% to 70% in preventing hospitalization from pneumonia, according to data obtained from a systematic review of 64 studies assessing the efficacy and effectiveness of influenza vaccine in people ≥ 65 years [5]. Age-related defects in the B cell compartment, such as the decreased generation of specific serum antibodies [55,56], switched memory B cells [55,57], and long-lived plasma cells [57] are in part responsible for sub-optimal antibody responses of elderly individuals to vaccination [22,57–59]. Several strategies have been applied to improve influenza vaccines for the elderly. These include the increase of the vaccine antigen from 15 to 60 μg of HA protein per dose [60], the administration of the vaccine by intradermal versus intramuscular route [61] and the formulation of the inactivated vaccine with oil-in-water emulsion adjuvants [62].

The high dose vaccine contains four times the amount of HA antigen compared to the traditional formulation and it has been associated with a stronger immune response and better effectiveness than the regular dose flu vaccine in older people [63], even though these responses do not achieve the magnitude of those induced by the standard dose vaccine in young adults [64].

Intradermal vaccination with trivalent IIV was licensed by the European Medicines Agency (EMA) for adults older than 60 years in winter 2010/11 in Europe, and in September 2010 in Canada. In 2011 it was also approved in the USA by the Food and Drug Administration (FDA) for subjects older than 64. Intradermal administration stimulates dermal population of specialized DC, such as Langerhans cells, that are extremely efficient in antigen presentation [65] and increases also the recruitment of DC and macrophage precursors from the blood stream. Mature DC migrate to the paracortical area of the draining lymph nodes, where they present processed antigens to T cells. Intradermal free antigen can also disseminate to the draining lymph node with consequent stimulation of resident DC and interaction with specific B cells. Results collected during the last few years, have demonstrated that intradermal influenza vaccines are able to confer a better immune response than TIVs at a full antigen dosage in the elderly and an equivalent response, at a lower antigen dose, in healthy adults and in patients with severe chronic diseases or immunocompromised [66].

The MF59-adjuvanted trivalent inactivated vaccine was specifically developed to increase the immune response of the elderly to influenza vaccination. MF59, a squalene-based oil in water emulsion described in detail in section 4.1, was first licensed in 1997 in Italy and later in more than 20 countries worldwide, in association with the seasonal inactivated subunit influenza virus vaccine for individuals aged 65 years or more. Its effectiveness and safety have been analysed in a large, prospective, randomized, observational study (2006–2009 influenza seasons, about 170,000 subjects) carried out in northern Italy that highlighted a 25% lower risk of hospitalization for influenza or pneumonia for the MF59-adjuvanted vaccine relative to trivalent inactivated vaccine [67,68]. Other emulsion adjuvants such as AS03 or AF03, based on squalene plus other components, have been licensed with the pandemic A/H1N1 vaccine and with the avian A/H5N1 vaccine, showing a higher efficacy for prevention of some subtypes of influenza than does a non-adjuvanted TIV [56,69–71]. Finally, vaccination of people that could come in close contact with elderly individuals, such as children and healthcare workers, can be considered as an indirect protection strategy.

Overall, studies evaluating the efficacy of influenza vaccination in the aged population are still controversial, and criticisms arise in the criteria used for evaluating efficacy and effectiveness [72]. Since randomized placebo-controlled clinical trials in this age class are very uncommon, most of the data arise from observational studies that have used different designs,

outcomes, and end points providing a large set of effectiveness estimates [73]. Confounding factors such as comorbidities, health status or previous history of vaccination can alter the estimates and different methods to adjust for these confounding factors have been used [74]. The efficacy findings analysed in younger, healthy seniors may not apply to older and frail seniors because of advanced age and the presence of serious medical conditions associated with immune functions decline.

3.2. Pneumococcal vaccine

Streptococcus pneumoniae is the most commonly isolated agent of community acquired pneumonia and also causes invasive pneumococcal disease (IPD), defined as the presence of the bacterium in a normally sterile site (blood or cerebrospinal fluid). Pneumococcal disease is most common at the extremes of age, with a steep increase in incidence in people over 65 years. The presence of co-morbidities and immunosenescence increase the susceptibility to community acquired pneumonia in general and to pneumococcal disease in particular [75]. Pneumococcal vaccination is therefore recommended in the elderly (Table 1). Licensed pneumococcal vaccines include the 23-valent pneumococcal polysaccharidic vaccine (PPV23) that contains 25 μg of purified pneumococcal polysaccharide of each serotype, and the 13-valent conjugated vaccine (PCV13) that contains 2.2 μg for each polysaccharide type, except 4.4 μg of serotype 6B, conjugated to the non-toxic mutant of diphtheria toxin CRM197 and 0.125 mg of aluminium phosphate as an adjuvant. PCV13 has been typically recommended for children under 2 years of age, while the PPV23 for adults and elderly. Recently, the use of PCV13 has also been recommended in the elderly [76] (https://vaccine-schedule.ecdc.europa.eu, https://www.cdc.gov/features/adult-pneumococcal/index.html), since PPV23 is poorly immunogenic, elicits a T-independent response and relatively low antibody titers with low functional activity. PPV23 has indeed shown low to moderate effectiveness against vaccine serotype pneumococcal pneumonia in people aged 65 years or older and this could vary by population groups [77]. Despite covering less serotypes, PCV13 is able to elicit a T-dependent response, producing high titers of functional (opsonophagocytic) antibodies and is thus being recommended in elderly populations. PCV13 has proven safe and immunogenic in the elderly even in the presence of comorbidities [76], but data on vaccine efficacy on this population are not yet available. Since the immune response to PPV23 is suboptimal in the elderly and repeated administration of PPV23 may also lead to hyporesponsiveness, vaccine strategies employing priming with PCV13 and boosting with either PCV13 or PPV23 are also recommended in the elderly population (https://vaccine-schedule.ecdc.europa.eu/; https://www.cdc.gov/features/adult-pneumococcal/index.html).

3.3. Herpes zoster vaccine

Herpes zoster (HZ, or shingles) is caused by reactivation of latent varicella zoster virus. Viral reactivation happens when components of cell-mediated immunity become compromised by disease, pharmacological treatments or aging. Incidence of HZ is in fact higher in the elderly and rises with age (3–5/1000 persons/year in the general population, 8–12/1000 persons/year in people > 80 years old) [78]. HZ is a major cause of hospitalization in the elderly (65/100.000 hospitalizations in people > 80 years old) and can be complicated by postherpetic neuralgia, with invalidating pain after the rash is resolved, or by eye involvement, when the ophthalmic branch of the trigeminal nerve is affected [78]. Two different vaccines against shingles are currently licensed: the HZ live attenuated zoster vaccine (Zostavax, Merck) and the subunit zoster vaccine (Shingrix, GSK). The live attenuated vaccine contains at least 20.000 PFU of the Oka strain, an attenuated VZV strain originally isolated in Japan that is also used in children, albeit at a lower dose, to prevent chickenpox. The recombinant subunit vaccine contains 50 μg of the VZV glycoprotein E (gE), which is a major component of the viral surface, formulated with the $AS01_B$ adjuvant (50 μg of *Quillaja saponaria* Molina, fraction 21 and 50 μg of 3-O-desacyl-4′-monophosphoryl lipid A from *Salmonella minnesota*, see section 4.1). The live attenuated vaccine was licensed in 2006 in the USA, the vaccine efficacy, assessed in a recent metanalysis, was 33% in preventing HZ, but it was higher in preventing both hospitalizations due to HZ (74%) and postherpetic neuralgia (57%) [79]. The recombinant vaccine has been licensed in 2017 and demonstrated an efficacy of about 97% in preventing HZ in 50 years of age or older adults, with a moderate reactogenicity (pain at injection site in 79.1% of recipients and myalgia in 46.3%) [80]. Immunological analysis revealed that the recombinant vaccine, containing $AS01_B$ adjuvant, elicits a robust and persistent memory response in older adults [81].

4. Next generation vaccines designed for the elderly

In this section we discuss the current limitations in vaccination of the elderly and promising routes that research can undertake to improve efficacy and effectiveness of this crucial medical, social and economic intervention. These include design of vaccine adjuvants specifically tailored for the elderly; inclusion of the concept of immunobiography in the development of new vaccines and aged patients' stratification; adoption of systems approaches to enable understanding and inclusion of complex phenotypes in vaccines design (Fig. 2).

4.1. Need for vaccine adjuvants

Adjuvants are substances capable of enhancing and properly skewing the immune responses to the vaccine antigen, and their choice can dramatically affect the type and the magnitude of the adaptive immune response to the vaccine antigen, by impacting on the innate response starting signal [82]. The presence of the adjuvant in the vaccine formulation can enhance the speed and magnitude of the development of immune responses, reduce the dose of antigen and/or vaccinations needed, increase cross-protection and reduce non-responsiveness in specific target populations such as the very young or the elderly. Development of vaccine adjuvants specifically designed to optimally stimulate the aging immune system, taking in consideration its inflammatory status and immunosenescence, is essential for the design of next generation vaccines for the elderly (Fig. 3).

Vaccine adjuvants currently used in the elderly are MF59 and AS03, included in influenza vaccines, and AS02 used for the recombinant HZ vaccine [62,83–85].

MF59 is an oil-in-water emulsion composed of squalene and the surfactants Tween 80 and Span85, firstly licensed as an adjuvant in a seasonal influenza vaccine for older adults in 1997 [62]. Even though the mechanism of action of this adjuvant is still not completely understood, MF59 was shown to induce the recruitment and activation of cells at the site of injection, to stimulate a local environment characterized by the expression of several immunostimulatory cytokines that can favour the uptake of the antigen by antigen-presenting cells and its transport to the draining lymph nodes [86]. Furthermore, an increased breadth of antibody response has been reported, demonstrating that MF59 influences both the quantity and the quality of anti-influenza antibodies [87]. Ongoing phase III clinical trials investigate MF59 also in a tetravalent influenza vaccine formulation in adults ≥ 65 years of age (NCT02587221; NCT03314662).

AS03 and AS01 belong to a family of adjuvants called Adjuvant Systems (AS), obtained by the combination of immunostimulatory molecules with classical adjuvants (such as aluminium salts, liposomes and oil-in-water emulsions), designed to provide better and broader protection than classical formulations [88]. AS03 has been licensed with pandemic A/H1N1 and avian A/H5N1 split inactivated monovalent vaccines, while AS01 with the recombinant malaria (RTS,S) and herpes zoster (RZV) vaccines. AS03 adjuvant contains squalene and the immunostimulant molecule α-tocopherol. Despite the fact that both MF59 and AS03 adjuvants are squalene-based emulsions, *in vivo* behaviours differ due to the presence of the other components. AS03 is able to strongly induce upregulation of genes encoding inflammatory cytokines and chemokines at distant sites, such as the draining lymph nodes, and this effect is specifically mediated by α-

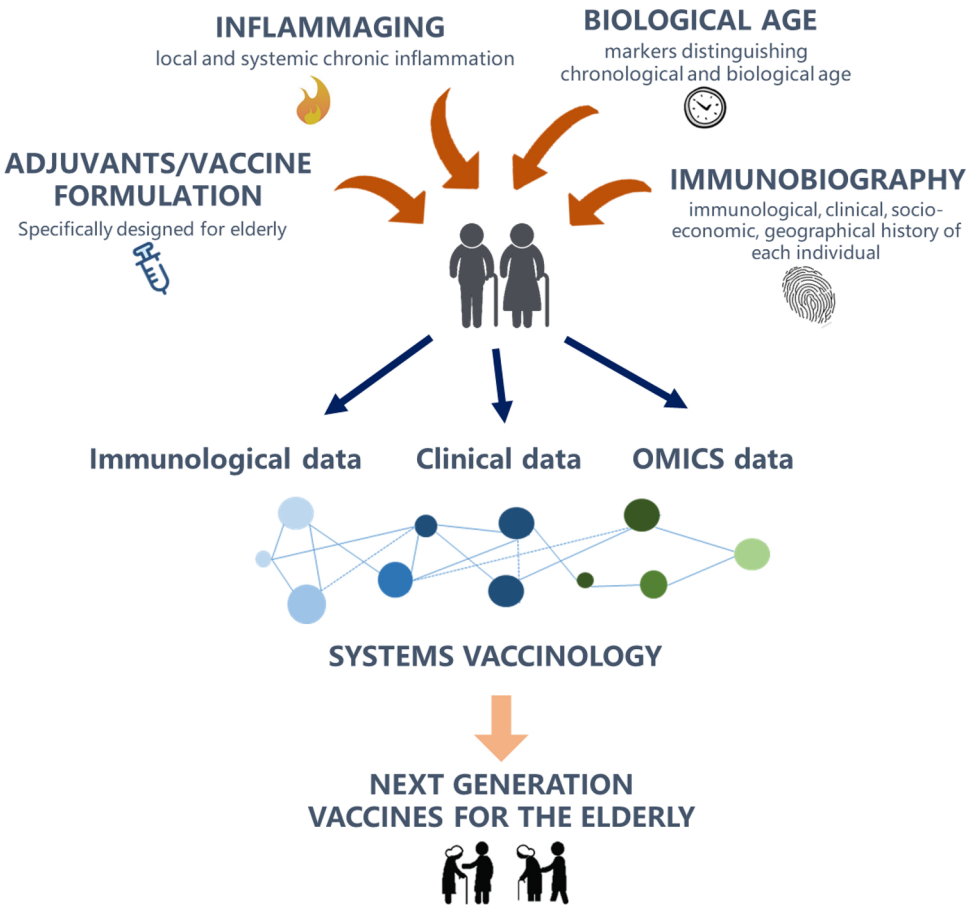

Fig. 2. Integrated strategy for the development of next generation vaccines for the elderly. The development of vaccination strategies for the elderly should consider an integrated approach based on the design of vaccine adjuvants/formulations with mechanism of action optimally suited to act in the context of an inflammatory status (inflammaging) taking in consideration the biological age and immunological history (immunobiography) of the elderly. The immunological, clinical and omics data generated from clinical studies of vaccination in the elderly should be integrated using a systems vaccinology approach to guide the design of next generation vaccines specifically tailored for the elderly.

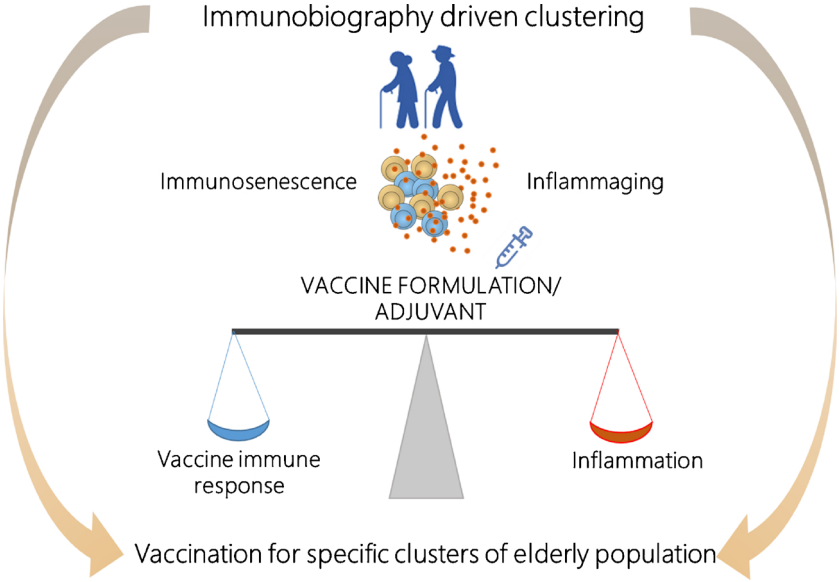

Fig. 3. Development of vaccine formulations adapted to the immune system of the elderly. Vaccine formulations, including adjuvants, should be specifically designed considering the immunological status of the elderly, in which a decreased immune responsivity due to immunesenescence, co-exists with the chronic, low-grade inflammation (inflammaging). The vaccine formulation/adjuvant should be designed to optimally balance between immune stimulation and inflammatory status. The immunobiography approach could inform the stratification of elderly subjects and guide the implementation of vaccination strategies designed for specific elderly population clusters.

tocopherol [89]. Studies comparing the adjuvanticity of the two squalene-based emulsion adjuvants combined with A/H1N1 monovalent vaccines, reported a higher humoral response with the AS03-adjuvanted vaccine associated with higher reactogenicity [90,91]. In order to reduce this side effect the AS03B, containing half a dose of α-tocopherol, has been developed and assessed in clinical studies [92].

AS01 is a liposome-based vaccine adjuvant system containing two immunostimulant molecules, the MPL (3-O-desacyl-40-monophosphoryl lipid A of *S. minnesota*), and QS-21, the lytic saponin fraction of QuilA. As described in section 3.3, the subunit HZ vaccine (Shingrix, GSK) contains the AS01 adjuvant combined with the gE glycoprotein of VZV. The unique ability of this vaccine to enhance a strong T-cell response has been demontrated in humans, where significantly higher T-cell and humoral responses are observed with adjuvanted compared to unadjuvanted vaccine antigen gE [93,94]. Large Phase III placebo controlled efficacy studies demonstrated high efficacy (> 90%) of the adjuvanted-vaccine in preventing herpes zoster in all studied age groups, including adults older than 70 years of age [83].

Profiling the mode of action of adjuvants is of critical importance, and many preclinical and clinical studies have been performed for assessing their interaction with both innate and adaptive immunity [95–104], however, limited studies have been focused on profiling the action of vaccine adjuvants in the elderly. A common characteristic of the different adjuvants in clinical use is an inflammatory signature in the first few hours after vaccination [105]. Deep knowledge of the mechanism of action of vaccine adjuvants and their behavior in the context of inflammaging should be considered in the design of vaccine formulations specifically tailored for the elderly [36]. Key open questions remain to be addressed to clarify whether the adjuvant should avoid increasing this inflammatory status or if more powerful adjuvants with strong inflammatory activities are needed to overcome this baseline low-grade inflammation status for eliciting effective immune response in the elderly. Ideally, the adjuvant should be designed to optimally balance between immune stimulation and the inflammatory status of the elderly immune system (Fig. 3).

4.2. Immunobiography

Immunobiography is a term dubbed in the original paper from Franceschi et al. [9] and refers to the comprehensive immunological, clinical, socio-economic and geographical history of each individual, able to account for the large heterogeneity observed in the elderly regarding their health status and immunological phenotypes mirrored by their large individual variation in the responsiveness to vaccines. The concept of immunobiography stems from inflammaging studies in particular regarding the identification of the possible inflammaging sources and of the causes leading to the number of different inflammaging phenotypes observed. Immunobiography postulates that old subjects' individual immunological inter-variability, including the inflammatory status, is the results of the lifelong exposures to external and internal immunological stimulations mediated by the genetic and epigenetic background, thus fully integrating the temporal dimension in the immunosenescence landscape. The immunobiography perspective addresses not only the individual aging process including the earliest years of life and antagonist pleiotropy effects, but also the historical perspective, integrating the profound changes that the anthropological environment has faced in the last 100 years.

It is possible to obtain individual immunobiography profiles by collecting appropriate informative data such as sex/gender, demographic and epidemiological history of the cohort, geography, individual immunological history, anthropometric parameters, socio-economic status and education, CMV sero-status, major morbidities and co-morbidities, genetics among others. Each of the above-mentioned parameters have proven to exert effects on the immunological phenotype of the elderly and on vaccination effectiveness, accordingly their combination should provide new valuable information applicable to a variety of clinical protocols. Age related physiological decline can be considered as a function of the *speed* or *rate of aging*, leading to a discrepancy between chronological and biological age [106]. Such discrepancy can be measured by aging biomarkers. An aging biomarker should well correlate with chronological age of individuals in the general population, and reflect the inter-individual variability in their aging rates, i.e. their biological age [107]. Several different markers and algorithms for the evaluation of biological age have been proposed such as: DNA methylation remodeling telomere length [108,109] and attrition [110], transcriptomics [111], routine clinical biochemical parameters [112], N-glycan quantification [113] and composite algorithms (mixing markers of different nature e. g. biochemical, anthropometrical and molecular) [114]. It is out of the purposes of the present manuscript to describe in detail such methods, but it is worth to mention that the results obtained from the application of such methods are encouraging since in most of the cases they were able to correlate with age related health traits [38] such as mortality [115,116], cognitive decline [117] and human progeroid syndromes [118]. However such methods need to be improved to be successfully applied in clinical applications since to date they fail to provide reliable individual information regarding the related risk of developing age related diseases [119]. Moreover, in a study from Belsky et al. [120], the authors studied eleven methods for evaluating biological age and the relevance of biological age as predictor of health parameters in the elderly in a longitudinal cohort of 964 middle-aged subjects. The results showed that none of the considered methods presented strong association with health-span associated phenotypes (balance, hand grip, physical limitations) but in many cases the different methods showed poor correlation among themselves, indicating that they catch different aspects of the aging process. Beside these limitations biological age evaluation is one of the most promising tools for the development of innovative clinical approaches to improve the health of the ever-growing elderly population. In particular it is of utmost interest to integrate such tools as a relevant parameter for the assessment of the individual immunobiography. To this regard it is worth to flag the paper from Bacalini et al. in this issue [121] that reviewed the literature linking inflammaging and age related epigenetic remodeling.

Sex and gender are also major determinants of aging health phenotypes and the consistent sexual dimorphism in life expectancy is a clear evidence of this, that influences infection susceptibility and vaccine response and efficacy in old subjects [122]. In general females show lower infection susceptibility and higher vaccination responsiveness, mainly due to the divergent and changing levels of sex steroid hormones that impact on immunity, inducing higher humoral and cell-mediated immune response to antigenic stimulation, vaccination and infections [122,123]. Animal studies indicate that hormonal replacement therapy exerts positive effect on vaccine efficacy, but this observation has to be reinforced by stronger epidemiological data [124,125]. Influenza vaccine responsiveness was observed to be in inverse correlation with testosterone, suggesting an immunosuppressive role for testosterone in the context of influenza vaccination [126,127]. Differences among sexes also exists in disease-specific vaccination rate and dosage is an important parameter. Nevertheless, despite the growing body of literature, sex and gender are still not taken into account in the design or dosing of the 65+ vaccination.

CMV is considered a major contributor of inflammaging and is a remarkable example of the impact of geographical parameters on inflammaging and immunosenescence since the prevalence of CMV infections vary significantly across populations [128]. CMV is generally asymptomatic in healthy individuals and 25–90% of the worldwide population is CMV seropositive, with higher prevalence in older adults [129]. The establishment of a latent infection by CMV is a common event likely correlated to immunosenescence by increasing the levels of highly differentiated effector memory cells in the CD8+ and CD4 + T-cell pools [15]. It has been shown that the high presence of specific T reg and T follicular helper cells during CMV infection appears to limit the efficacy of influenza vaccine in older people, being less capable to help B cells when faced with new antigens [130]. Other phenomena may be at work like increased levels of cytokines in B cells and diminished B cell function that predicts poor antibody responses to other viral vaccination [59], with still controversial conclusions.

To manage the heterogeneity of elderly physiology and pathophysiology, geriatric medicine identified a series of syndromes that allow to

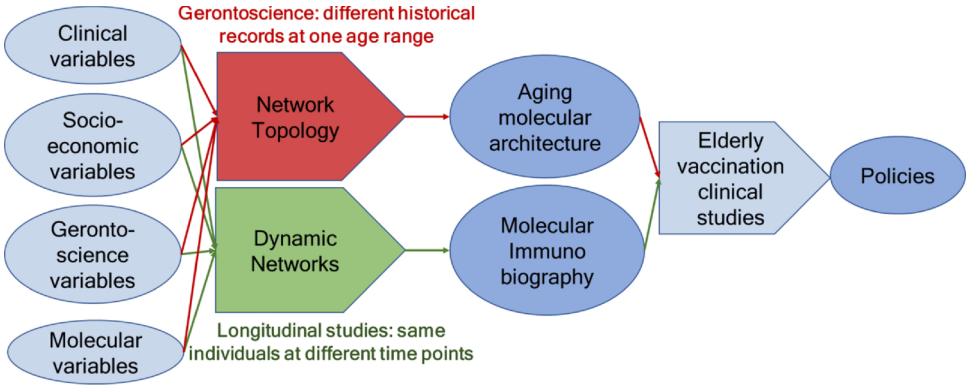

Fig. 4. Impact of systems vaccinology in the process from clinics and sciences of aging to policies for elderly vaccination. Systems vaccinology can exploit retrospectively (and here lies one of its strengths) data from two major sources of clinical studies: studies of aging where the fixed variable is chronological age, and longitudinal studies where individuals (fixed variable) are followed-up with molecular screens (ideally multi-omics) across a long age span. A variety of routine or ad hoc information could be available for both types of studies, including clinical and socio-economic variables. Topological network analyses as well as temporal simulations can be used to analyse such data and extract information meaningful in the design of novel vaccination clinical studies on the elderly.

classify the elderly population according to the relative risk of developing different diseases. These include metabolic syndrome, chronic obstructive pulmonary disease and mild cognitive impairment, frailty.

Vaccination in the elderly has to take in consideration a delicate balance between immunosenescence, which makes the elderly less responsive to vaccination, and inflammaging. The vaccine formulation and adjuvant used should be designed to optimally stimulate the elderly immune system inducing effective immune responses without exacerbating the inflammatory status. Application of the immunobiography approach could inform the stratification of elderly subjects and guide the implementation of vaccination strategies designed for specific elderly population clusters (Fig. 3).

4.3. Systems vaccinology to inform the development of vaccines tailored for the elderly

Vaccinology can benefit from the recent advances in systems biology, moving towards a better understanding of complexity. This evolution is possible and necessary, today, owing to the unprecedented dimensionality that molecular data have acquired in the *omic* era: thanks to enabling high-throughput technologies, virtually all elements of a given molecular layer can be qualitatively and quantitatively measured in one or a parallel series of *omic* experiments. This has dramatically changed the approach to the analysis of experiments. *Omic* studies record the activity of each molecule of interest in large tables with thousands of rows (molecules) and tens of columns (individuals/patients/conditions), that require automated mathematical and statistical approaches to be made sense of.

A very powerful mean to achieve this is the use of networks that can represent not only large number of variables as nodes, but also the relation among these variables, as edges. Indeed, such a flexible frame is well adapted to the needs of systems vaccinology as it allows to integrate gene expression and other *omic* data with clinical and immunological readouts to identify stable and robust markers of vaccine response.

This approach was first applied for the characterization of the human response to the live attenuated yellow fever vaccine 17D (YF-17D), providing the proof-of-concept evidence of the capacity of systems approaches to delineate “molecular signatures” predictive of vaccine responses [131,132]. Systems vaccinology has since been applied to characterize the immune response to a multitude of vaccines, including live attenuated and inactivated seasonal influenza [95,133–135], meningococcal vaccines [136], shingles [137,138], malaria [139–141], smallpox [142], Ebola [143–146] and HIV [147] vaccines. Additional work has been done to identify a variety of gene signatures in blood able to accurately classify and further predict vaccines responsiveness [136,148]. These studies have been conducted in young healthy adults and children but not in elderly population.

The application of this approach to study vaccination in the elderly could take advantage of increasing network heterogeneity by adding markers of aging. These include biological age and inflammaging, chronological age, and also personal immunological history, socio-economic variables, as well as clinical parameters, like the ones defining metabolic syndromes, chronic obstructive pulmonary disease, cognitive impairment, frailty. Knowing that medical and socio-economic variables have, at least in part, molecular correlates [149,150] often associated to epigenomic characterization (see review on the epigenetics of inflammaging in this issue [114]), systems vaccinology can define and further take advantage of very rich molecular networks. On such complex networks, mathematical tools can then operate to improve our predictive ability in vaccinology in the elderly, in two main respects.

One explores the structure of the network, i.e. its topological characteristics (the relative position of nodes and edges in the network). For example, nodes connected by a large number of edges (hubs) often represent essential components of the system; tightly packed sets of nodes (clusters) represent variables associated in a biological activity; nodes that are at the crossroad of several paths (chained sets of nodes characterized by high stress and betweenness) are potential critical targets [146]. Such structures therefore are helpful in elucidating the relevant molecular signatures of vaccine responses in the elderly. For this approach, all possibly relevant nodes should be included in the network, like the ones collected in large gerontoscience records (Fig. 4 top panel).

The second one exploits the fact that networks can also describe temporal evolutions: edges represent a status change of the variables represented in the nodes. Mathematical tools for this application span from the usage of Markov chains to Petri and Bayesian networks to the integration of topological information [149]. The ensemble of the nodes (states) before and after the transition(s) can be used to simulate or describe a sequence of events (health trajectory) and understand how present and past events impact on future responses, for this longitudinal studies are necessary (Fig. 4 bottom panel). Ensembles of future states can be grouped to define types of response to vaccination, and the initial conditions can be used to define better criteria for patients’ stratification. The complementarity and redundancy of the information gained with these two approaches can serve to the enhanced design of vaccination studies in the elderly based on informative enrolment criteria and whose result can be converted in effective vaccination policies.

5. Conclusions

The increasing life expectancy combined with decreasing birth rates leads to the increase of aged population worldwide. Elderly are vulnerable to illnesses, hospitalizations, and deaths that could be prevented through the

use of vaccines, recommended in many high-income countries. Nevertheless, the efficacy of vaccination in the elderly is strongly reduced compared to younger adults, mostly due to alteration of their immune system, where some immunological components are declined while others, such as inflammation, are increased. Most of the available vaccines are designed for a young population; there is the need to design vaccine formulations, including vaccine adjuvants, that optimally stimulate the elderly immune system taking in consideration the inflammaging component. Moreover, the specific history of each individual (immunobiography) that accounts for the large individual variation in the responsiveness to vaccines should be considered.

A systems vaccinology approach, incorporating the concepts of immunobiography integrated with clinical, immunological and *omic* data, has the potential to contribute to the stratification of subpopulations and identification of markers that will guide the rational development of vaccines specifically designed for the elderly. Deeper understanding of optimal strategies to stimulate elderly immune system would have an enormous impact not only in the optimization of existing vaccines but also to guide the development of novel vaccines highly needed for the elderly.

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