

Minireview

Vaccination in the elderly

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Summary

There is a general consensus that the elderly do not respond as well to vaccination as the young, but robust studies are few and far between. Most refer to influenza vaccination, but even here, adequate immunological and clinical data are surprisingly thin on the ground. The meta-analysis by Goodwin *et al.* from 2006 is still the most comprehensive that we have. They reviewed 31 antibody response studies comparing influenza vaccination efficacy in groups of elderly and younger adults. They reported that the adjusted odds ratio (OR) of responses in elderly versus young adults ranged from 0.24 to 0.59 for the three influenza antigens used in the vaccines. They concluded that rather than the estimated 70–90% clinical vaccine efficacy in younger adults, this figure was only 17–53% in the elderly, depending on which viruses were prevalent that year. They stated that ‘this highlights the need for more immunogenic vaccine formulations for the elderly’. How to achieve this? There are three areas where we may consider alterations to increase vaccine efficacy: (i) make the vaccine more potent; (ii) use adjuvants to enhance immunity; and (iii) apply immune modulators or other interventions to alter host immunity generally. We will consider these three options, focusing on influenza vaccination, in this mini-review.

Introduction

Vaccination commonly means active vaccination in which immunogenic materials from pathogens (or tumours) are

administered to the host who is required to generate an adequate immune response specifically to these antigens. In most countries, it is recommended that all elderly people receive seasonal influenza vaccination annually (and vaccination against other pathogens according to their likelihood of exposure, e.g. travel vaccines). The efficacy of vaccination is not monitored routinely for the individual patient. In establishing vaccine efficacy for licensing purposes, often only younger subjects are tested and usually only antibody titres are measured. This is because it is assumed that because these vaccines are given prophylactically, sterilizing antibody titres must be reached to prevent *de novo* infection. Thus, each year, seasonal influenza vaccine is licensed solely according to serological criteria, which were originally established as protective in younger individuals. Even when elderly individuals are assessed as having responded to the vaccine in terms of antibody production, data are lacking on how well these people are actually protected against disease. B cell responses for influenza antibody generation require that CD4 T-helper cells are activated by the vaccine, but do not take into account whether CD8 cytolytic T cells have been activated as well. However, where CD8 activation has been measured, it has been reported to correlate better with clinical protection than antibody titres (McElhane, 2011). This may be because the antibody levels are not 100% protective against infection, and CD8 effector cells are then required to lyse infected host cells. Therefore, the requirements for a successful vaccine (Table 1) are that (i) vaccine antigen must reach and be picked up and presented by dendritic cells (DC) or other antigen-presenting cells (APC); (ii) the DC must be able to activate and facilitate the functional differentiation of CD4 helper T cells and preferably CD8 cytotoxic T cells as well; (iii) B cells must be able to respond to the vaccine components and must be susceptible to help from CD4 cells for production of mature antibody; (iv) regulatory controls that damp down immune responses should not be stimulated to overactivity by the vaccination process.

However, almost all of these components are altered with age. In humans, one major subset of DC, the plasmacytoid DC (of lymphoid origin), are present at lower numbers in the aged, and appear functionally compromised in that they secrete lower amounts of type I

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Table 1. What are the requirements for vaccination which are compromised in the elderly?

Vaccination requirement	Status in the elderly
Vaccine antigens must be presented by APC	APC function may be compromised
Helper T cells must be activated	CD4+ T cells are susceptible to ageing
B cells must be able to produce antibody to prevent infection	T cell help as well as intrinsic B cell physiology is altered with age
Cytotoxic T cells must differentiate to kill infected cells and cure infection	CD8 cells are the most severely affected component of immunity in the elderly

interferons, whereas the data on 'conventional' myeloid-derived DC are disparate. Some studies suggest that the numbers of cDC in the elderly are lower than in the young and they tend to have a more mature phenotype as would be expected had they already encountered and been stimulated by antigen in the past (Della Bella *et al.*, 2007). This is in fact a recurring theme for immune responses in the elderly in general: different individual immunological histories shape current ongoing responses, which may explain some of the disparities in the literature. On the other hand, several studies reported that antigen uptake, processing and presentation by blood DC, is quite well-retained in the elderly, including findings that young and elderly DC stimulate naïve CD8+ T cells equally well, which would be good for triggering cytotoxic responses to influenza vaccines (but note that the elderly commonly possess far fewer naïve cells than the young, and that much of the anti-influenza vaccine response is likely to be a memory response). However, it was noted that DC from the elderly stimulate naïve CD4+ T cells less well (Agrawal *et al.*, 2008; Agrawal and Gupta, 2011). Altered proportions of different differentiation stages of DC might be partly responsible for this, helping to explain cytokine production differences in the elderly. These are also reflected in different surface molecule expression and function in old DC, especially the Toll-like receptors (TLR) essential for initial DC activation, as well as decreased expression of T cell stimulatory molecules (CD80, CD86) and increased expression of co-inhibitory molecules (e.g. PD-L1). These differences influence the type of naïve T-cell response triggered by these DC. In the context of influenza vaccination, a direct correlation between lower levels of CD80 and CD86 and subsequent responses to the vaccine has been reported (van Duin *et al.*, 2007). For a detailed discussion of the current state of the art in this area, the interested reader is directed to an excellent recent review on the impact of ageing on DC in humans by leaders in the field (Agrawal and Gupta, 2011).

The next step after uptake of the vaccine antigen by the DCs is the activation of T cells. Also T cells, specially CD8+ T cells are susceptible to age and ample evidence from mainly cross-sectional but also few longitudinal

studies demonstrate a strong difference in the T-cell compartment between young and old individuals. Both the distribution of different subsets and functional properties on a per-cell basis are altered during aging. The main age-associated changes include: (i) decreased numbers and clonal diversity of naïve T cells in the periphery; (ii) reciprocal accumulation of late-differentiated memory T cells, which may have reduced functionality and clonal diversity; and (iii) altered T-cell signalling (Goronzy *et al.*, 2007; Larbi *et al.*, 2008). These changes at the population as well as the cellular levels can lead to an altered cytokine production profile of T cells as well as altered requirements of these cells for proper activation by professional APCs in the elderly, which can in turn have an impact on the outcome of influenza vaccination (Deng *et al.*, 2004; Corsini *et al.*, 2006).

Age-associated changes to B cells, the main players in a vaccine-induced humoral immune response are less-well studied and the available data are inconsistent. However, they suggest a reduced B-cell diversity and function in the elderly and, as with T cells, the reduced frequency of naïve B-cells has been proposed as a hallmark feature of immunosenescence (Colonna-Romano *et al.*, 2008). At the cellular level, age-associated alterations in immunoglobulin generation during immune responses (through class switching and somatic hypermutation) in B cells are observed (Frasca *et al.*, 2005), which may also contribute to the decline of the quality of humoral response in the elderly. The clinical implications of intrinsic age-associated defects of B-cells in the context of flu vaccination in the elderly were demonstrated in a recent study (Frasca *et al.*, 2010).

Finally, regulatory mechanisms dampening an immune response might also be differentially regulated between young and elderly people. CD4+ regulatory T cells (Treg) expressing the transcription factor Foxp3, are one of the main regulatory subsets, involved in maintaining self-tolerance and control of autoimmunity, through suppression of activation, proliferation and cytokine production of T cells (Sakaguchi *et al.*, 2010). Several studies have shown an increased percentage of regulatory T cells in the elderly (Gregg *et al.*, 2005; Trzonkowski *et al.*, 2006; Vukmanovic-Stejic *et al.*, 2006; Rosenkranz *et al.*, 2007; Lages *et al.*, 2008), whereas one recent study failed to detect any difference between the frequency of Foxp3+ T cells between young and old individuals (Hwang *et al.*, 2009). As far as the function of these cells is concerned, decreased (Tsaknaridis *et al.*, 2003) or un-altered (Gregg *et al.*, 2005; Trzonkowski *et al.*, 2006; Vukmanovic-Stejic *et al.*, 2006; Rosenkranz *et al.*, 2007; Hwang *et al.*, 2009) suppressive capacity of regulatory T cells in the elderly has been reported. Thus, although the function and homing properties of Tregs is retained during aging (Lages *et al.*, 2008; Hwang *et al.*, 2009), the increased

levels of this suppressive population would probably lead to a higher suppressive effect in this group possibly contributing to the decreased immune function and response to flu vaccination observed in the elderly (Goodwin *et al.*, 2006).

Better vaccines

Earlier attempts to increase efficacy of vaccination in the elderly simply employed larger doses of antigen, and this seems to be effective to some degree (Remarque *et al.*, 1993; Keitel *et al.*, 2006; Cate *et al.*, 2010; Fiore *et al.*, 2010). That is, antibody titres tended to be increased, but it was not proven that this actually correlated with clinical protection. These data are currently being collected. The first commercial influenza vaccine designed specifically for use in the elderly entered routine practice only for the 2010/2011 influenza season. The main innovation was to use a specially designed injection system for easy intradermal (i.d.) delivery of a (double dose) of the regular three-component influenza vaccine. It is not yet clear whether superior clinical efficacy will be attributable to this innovation either. However, from the theoretical point of view, at least it seems more sensible to inject vaccine antigens into an area replete with skin DC, rather than into the muscle (i.m.) as is traditional. Nonetheless, although not studied in great detail, reports of fewer and less active skin DC (Langerhans cells) in the elderly than the young (Grubeck-Loebenstein *et al.*, 2009) do suggest that even i.d. vaccination will not be as effective in older as in younger people.

Many other attempts to improve the vaccines used in the elderly have been made over the years, such as the use of combinations of nasally delivered live attenuated virus and inactivated viral components, instead of inactivated virus alone. One trial reported an enhanced response in the elderly (Treanor *et al.*, 1992), but this has also not been followed up. Virosomal vaccines, inactivated but reconstituted viral particles, have also been tested in the elderly, possibly with advantage (de Bruijn *et al.*, 2006). One recent area that is attracting a great deal of interest is to target less variable influenza antigens and to stimulate more CD8 T-cell activity (Berthoud *et al.*, 2011); this approach remains to be tested in the elderly.

Better adjuvants

Considering the age-associated defects in dendritic cells discussed above, better vaccines automatically beg the question of how to improve DC function, which is the job of adjuvants. Adjuvants are substances injected together with the vaccine antigens, which are designed to enhance the immune response. Most influenza vaccines in most countries are unadjuvanted or adjuvanted only with alum,

which is not very effective. Limited clinical trials using variations on the theme of oil in water emulsions, canonically incomplete Freund's-type adjuvants, such as MF59, documented enhancement of antibody production, even when used i.m. (Clark *et al.*, 2009; Galli *et al.*, 2009). However, efficacy might be expected to be much higher when adjuvanted vaccines are given i.d. rather than i.m. but this was not tested. This adjuvant has also been tested for safety in the elderly (De Donato *et al.*, 1999; Minutello *et al.*, 1999), but T-cell responses seem never to have been studied. Thus, much work remains to be done. More work on adjuvants has already been done in the field of cancer vaccination, but here the concerns regarding side-effects are less pressing. More sophisticated adjuvants targeting TLR may be more effective in specifically activating DC for antigen presentation. In particular, combinations of TLR agonists could be especially effective, as demonstrated in animal models of lung infections where treating with ligands to TLR2/6 and TLR9 appeared to result in synergistic effects on protection (Duggan *et al.*, 2011). Since these TLR transmit activating signals to DC via a common MyD88-dependent pathway, direct pharmacological targeting of that pathway could also be effective in enhancing DC activity if age-associated deficits in TLR expression or function were present. Certainly, multiple deficits in DC signal transduction pathways required for optimal activation have been described in the context of ageing (for review see Agrawal *et al.*, 2008).

Although adjuvants commonly act on APC, other cell types may also be targeted (although perhaps strictly one should rather speak of immunomodulation in this case). At least the immunomodulatory glycolipid α -galactosylceramide (α -GalCer) is usually classed as an adjuvant. This agent is a ligand for the small subset of T cells carrying the minimally variant $\gamma\delta$ chain receptor rather than the astronomically diverse $\alpha\beta$ receptor, as well as for so-called NKT cells. Both of these cell types recognize α -GalCer presented by the non-classical MHC-like CD1 surface molecule on APC. NKT cells stimulated in this manner may enhance responses to influenza vaccination, at least in a mouse model (Guillonnet *et al.*, 2009). Although aged humans also have deficits in NKT cells (DelaRosa *et al.*, 2002), stimulation targeting them specifically might still be beneficial.

Better immunity

Enhancing immunity in the elderly requires a careful consideration of what is appropriate and what might be damaging. Of the large number of immune-related factors which have been reported as different in the young and elderly, it remains unclear which are detrimental (thus contributing to 'immunosenescence' and targets for manipulation) and which might be neutral or

even adaptive (thus potentially dangerous to manipulate). Here, we are really suffering from a lack of data. Longitudinal studies of aging cohorts correlating 'biomarkers of aging' with health outcomes would be ideal for this purpose. However, these are expensive, complex and difficult to organize in human cohorts. Animal models may tell us something, but it is likely that given the evolutionary differences between mice and men, it will be necessary to study the appropriate species. Many longitudinal studies are ongoing, but unfortunately few include immunology or even any type of biomarker analysis. One series of such studies of the very elderly > 85 years of age (the Swedish OCTO/NONA-Immune) identified a simple cluster of immunological parameters predictive of 2-, 4- and 6-year mortality at follow-up (Wikby *et al.*, 2005). The main factors were all related to an accumulation of late-differentiated CD8 cells, which resulted in an inverted CD4 : CD8 ratio, high levels of CD8 + CD28-negative cells and poor proliferative responses. Lower numbers of B cells were also part of this so-called 'immune risk profile' (IRP), which included infection with the persistent human herpesvirus HHV5 (Cytomegalovirus) (Pawelec *et al.*, 2009). Although there were no data on the outcome of influenza vaccination in the OCTO/NONA studies, independent reports have suggested that the presence of large numbers of CD8 + CD28-negative cells (Goronzy *et al.*, 2001; Saurwein-Teissl *et al.*, 2002; Xie and McElhaney, 2007) and seropositivity for CMV (Trzonkowski *et al.*, 2003) both correlate with poorer responses to vaccination. These alterations may all be consequent upon the fact that CMV infects and resides in antigen-presenting cells themselves and is therefore in a prime position to affect multiple parameters of adaptive immunity including downregulating DC stimulating capacity (Varani *et al.*, 2009).

From all this, one obvious opportunity to enhance appropriate immunity to influenza vaccination would be to employ anti-CMV strategies (Derhovanessian, 2010). However, available pharmacological agents are not without side-effects, and are not terribly effective either. It would be hard to justify using them in the overtly healthy elderly. Vaccination approaches would be difficult, as they would need to be therapeutic, not prophylactic and anyway the immune response to CMV is already so great that it appears that it itself may be part of the problem; additionally, there has been limited interest in making a CMV vaccine and only recently has any success been reported (Pass *et al.*, 2009).

As alluded to above, a large body of experience in using active vaccination in a therapeutic context has accrued in the cancer immunotherapy field. Here, unlike with influenza vaccination, the use of adjuvants to enhance responses is commonplace, as discussed in *Better immu-*

nity section. What has in addition recently greatly excited the cancer immunotherapy community has been the use of agents, which influence the control of the immune response, especially the results of trials with the very recently FDA-licensed anti-CTLA-4 (CD152) antibody ipilimumab ('Yervoy') (Ledford, 2011). The target of this blocking antibody is a CD28-like surface receptor expressed on activated T cells which also binds the CD28 ligands CD80 and CD86, but with higher avidity. CD28 co-stimulates T cells and is downregulated thereafter; CD152 in contrast is upregulated only on activated cells and transmits a modulatory downregulating signal. Blocking the interaction of CD152 with its ligands releases the 'brakes' on immune responses. This has led to some favourable outcomes in treating cancer, but also with sometimes severe autoimmune side-effects (Weber, 2009). It is therefore unlikely that it could ever be used in the elderly to enhance influenza vaccine responses. However, there is a whole group of families of co-stimulatory and co-inhibitory receptors and their respective ligands as well as CD28 and CD152 (e.g. ICOS, 4-1BB, PD-1, etc.), and judicious modulation of these might be able to enhance responses without undue risk of autoimmune disease. Other manipulations to enhance immune responses that have been explored in the cancer vaccination field might one day also be applicable to the elderly without cancer, including cytokine and anti-cytokine therapy also used in other disease states. In fact, an earlier study reported enhanced responses of the elderly to influenza vaccination under low-dose IL 2 therapy (Provinciali *et al.*, 1994), but these studies have been discontinued due to regulatory issues. Similarly, earlier trials reporting the superior efficacy of IL 2-supplemented liposomal influenza vaccines in the elderly (Ben-Yehuda *et al.*, 2003) have not entered routine clinical practice. Also depletion of Tregs has proven successful in enhancing vaccine-induced responses in the field of cancer immunotherapy (Dannull *et al.*, 2005; Mahnke *et al.*, 2007) as well as anti-viral responses in animal models (Furuichi *et al.*, 2005; Chuang *et al.*, 2009). Considering the increased levels of this suppressive population observed in the elderly discussed above, this approach might indeed improve the outcome of vaccination in this group.

Given the generally higher baseline pro-inflammatory state in the elderly, and the notion that this contributes to frailty, morbidity and mortality reported in countless studies, the application of anti-inflammatory agents may well be beneficial also for responses to influenza vaccination. In one study from 1994, this was indeed found to be the case (Hsia *et al.*, 1994), but this does not seem to have been followed up. Also stress has been associated with a poorer outcome of flu vaccination (Vedhara *et al.*, 1999). Consequently stress management measures were shown to enhance antibody responses in the elderly

Box 1. How to improve appropriate immunity in the elderly?

- **Reduce source of chronic antigenic stress** (eg. anti-virals, anti-cancer treatment)
- **Reconstitute thymic function** (eg. IGF, KGF, IL 7)
- **Improve B cell production** (eg. IL 16)
- **Reconstitute naïve repertoire** (eg. use cryopreserved naïve or culture-expanded naïve T cells)
- **Block inhibitory receptors** (eg. antagonistic Abs)
- **Maintain CD28 expression** (eg. neutralise TNF- α)
- **Maintain telomerase expression** (eg. TA-65)
- **Provide appropriate milieu** (eg. IL 7, IL 15)
- **Reduce proinflammatory status** (eg. Aspirin, anti-IL 6)

receiving flu vaccination (Vedhara *et al.*, 2003). A general, speculative, non-exhaustive list of possible interventions to restore and improve appropriate immunity in the elderly, which would also be expected to enhance responsiveness to vaccines, is given Box 1. It would be out of place to discuss all these possibilities here; suffice it to say that all of the approaches listed in the table have or are being studied at some level and might be appropriate for translation at a later date. For a more detailed discussion of these possibilities, the interested reader is directed to reference Fülöp and colleagues (2007).

Concluding remarks

Vaccination of older adults is in general not as effective as in younger people, but robust data on such an important topic are still inadequate, and the reasons for poorer responsiveness are unclear. Dissecting the factors influencing 'immunosenescence' in this context represents an important public health issue because of the increasing numbers of elderly people and their greater susceptibility to infectious disease. Improved vaccination approaches, likely focusing on better adjuvants in the short term, will have profound implications for the health and wellbeing not only of the elderly, but also of the 'herd' to which they belong.

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