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CD4 T cell Defects in the Aged: Causes, Consequences and Strategies to Circumvent

Wenliang Zhang¹, Vinayak Brahmakshatriya¹, and Susan L. Swain*

Department of Pathology, University of Massachusetts Medical School

Abstract

Aging leads to reduced immunity, especially adaptive responses. A key deficiency is the poor ability to mount robust antibody response. Although intrinsic alterations in B cells with age are in part responsible, impaired CD4 T cell help makes a major contribution to the poor antibody response. Other CD4 effector responses and memory generation are also impaired. We find delayed and reduced development of CD4 T follicular help (Tfh) cells in aged mice in response to influenza infection with reduction of long-lived plasma cells. When we examine CD4 subsets we also find shift towards Th1 and cytotoxic CD4 (ThCTL) responses. We summarize strategies to circumvent the CD4 T cell defect in aged, including adjuvants and proinflammatory cytokines. We find that we can strongly enhance responses of aged naïve CD4 T cells by using Toll-like receptor (TLR) activated dendritic cells (DC) as APC *in vivo* and that this leads to improved germinal center B cells and IgG antibody responses. The enhanced response of aged naïve CD4 T cells is dependent on IL-6, production by the DC.

Keywords

Aging; CD4 T cells; CD4 T cell help; Ab production

1 Introduction

One distinguishing feature of aged CD4 T cells is an increase in memory phenotype cells in both humans and in mice and in human CD4 T cells. During the normal aging process, the T cell output from thymus gradually diminishes over time, resulting in fewer naïve and increased memory phenotype CD4 T cells. This progression is independent of pathogen and antigen exposure in aged TcR transgenic (Dobber and others 1992; Sprent and Surh 2011), as well as in germ-free mice (our unpublished observation). With age naïve CD4 T cells become longer-lived (Tsukamoto and others 2009; Tsukamoto and others 2010), and in humans undergo increased homeostatic division (Nikolich-Zugich 2012), both of which

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*Corresponding author at: Department of Pathology, University of Massachusetts Medical School, Worcester MA 01605, USA Tel: 508-8564494, Susan.Swain@umassmed.edu (Susan L. Swain).

¹These authors contributed equally

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cause them to progressively develop intrinsic changes that reduce their response to antigen (Tsukamoto and others 2009). Accumulated exposure to antigen and inflammation with time can lead to clonal expansions of CD8 T cells further depleting the naïve repertoire (Yager and others 2008).

These deficiencies in both CD4 and CD8 T cell immunity contribute to reduced and short-lived responses to vaccine in the elderly population and reduced responses when they first encounter a new strain or pathogen, rendering them vulnerable to rapidly evolving infectious diseases such as influenza, SARS and to other emerging pathogens.

2 Impaired T cell help to B cells

One key problem is the poor ability of the aged to mount robust antibody responses that is likely due to the combination of both T- and B-cell intrinsic defects. Aged mice develop low levels of antibody with reduced duration of protective immunity following immunization (Steger and others 1996; Weksler and Szabo 2000). We have tracked the influenza-specific total IgG, IgG1 and IgG2b as well as IgM from 14 to 100 days post A/PR8/34 influenza infection. Although the levels of IgM are equivalent in young vs. aged mice, the total IgG and all the IgG isotypes are significantly 10-100-fold lower. The defects in IgG responses are in part due to intrinsic alterations in B cells with age, including class switch recombination (CSR) defects due to decreased induction of E47 and of activation induced cytidine deaminase (AID) (Cancro and others 2009; Frasca and others 2008) and/or an intrinsic VH repertoire shift (Klinman and Kline 1997; LeMaout and others 1997). Impaired CD4 T cell help is also likely to be partly or largely responsible for the poor B cell antibody responses (Eaton and others 2004). There is a delayed and reduced development of CD4 T follicular help (Tfh) cells in aged mice during A/PR8/34 influenza infection (our unpublished data), which is consistent with impaired and delayed germinal center (GC) formation. We find that there is a significant reduction of long-lived plasma cells (LLPC) in aged mice, most likely a consequence of the defective Tfh development. The detailed mechanism awaits for further investigation.

3 A shift in CD4 subset potential

We find that with age naïve CD4 T cells become less responsive to IL-6 signaling and this reduces their ability to become Th2/Tfh effector subsets, each of which provide helper functions. However, naïve CD4 T cells from aged mice still make Th1 and cytotoxic CD4 (ThCTL) effector subsets, producing an intrinsic change to a more inflammatory/cytotoxic response compared to that of young CD4 T cells. ThCTL is a unique subset of CD4 effector T cells that are cytotoxic and which can provide protective role during viral infection killing Class II expressing targets and/or secreting cytokines and chemokines that promote repair (Brown and others 2012; Marshall and Swain 2011; Mucida and others 2013; Strutt and others 2012). Our lab has identified ThCTL population during influenza infection, which peak around day 8 in young mice. The peak ThCTL is delayed by 5 days in aged mice but is of higher magnitude. What contributions these changes make to protective immunity mediated by CD4 T cells is not yet defined.

4 Vaccine adjuvants to enhance aged immune responses

Adding adjuvants to vaccines has been shown to enhance the host immune responses. Adjuvanted influenza vaccines induce stronger and more effective serologic responses in the elderly than conventional non-adjuvanted vaccines, not only against homologous but also against heterologous strains (Del Giudice and others 2006; Podda and Del Giudice 2003), supporting the notion that better vaccines can be designed that can overcome poor vaccine-induced protection in the elderly population. Most of the known approved vaccine adjuvants such as, bacterial endotoxin, monophosphoryl lipid A, bacterial CpG motifs, MF59 and a-galactosylceramide boost immunity by activating dendritic cells (Lambrecht and others 2009) via pattern recognition receptors, that recognize pathogen motifs including Toll like receptors (TLR). However, adjuvants act pleiotropically on most cells of the immune system and their effects are not restricted to pathogen-specific cells. Thus, in almost in all cases, increased adjuvant potency is associated with increased reactogenicity and toxicity, which includes local reactions such as inflammation, and swelling, to granulomas, lymphadenopathy, and ulceration (Petrovsky 2007) or more systemic reactions such as fever, Uveitis, arthritis (Allison and Byars 1991). This is also true for the influenza adjuvant MF59, a squalene based adjuvant that is associated with major increases in injection site pain and reactogenicity (Frey and others 2003). Another concern with squalene oil is its ability to induce chronic inflammatory arthritis in susceptible animal models (Carlson and others 2000; Petrovsky 2007). The fact that adjuvants stimulate widespread systemic inflammation and cause fever and end-organ damage in septic patients, the most common victim of which are the elderly (Lien and Golenbock 2003) is a major drawback precluding their widespread use.

5 Inflamm-aging and role of proinflammatory cytokines in enhancing CD4 T cell responses

Despite functional defects in innate immune cells on a per-cell basis in the elderly, inflammatory processes of uncertain origin occur ubiquitously with increasing age, termed as "Inflamm-aging" with increased levels of pro-inflammatory cytokines IL-1, TNF α and IL-6 in serum (Franceschi and others 2005) (Weinberger and others 2008). Paradoxically, these very pro-inflammatory cytokines enhance the aged immune response when administered along with purified antigens. Studies by Haynes and colleagues have shown that combination of the cytokines Tumor Necrosis Factor α (TNF α), IL-1 and IL-6 enhance aged naïve CD4 T cells responses including their *in vitro* and *in vivo* proliferation and IL-2 production and also their ability to help B cells (Haynes and others 2004; Haynes and others 1999; Maue and others 2009).

One possibility is that persistent low-grade inflammation may reduce the ability of immune cells to respond to "danger" signals induced by pathogens or vaccines. It seems reasonable to conclude from observations of others (Goronzy and Weyand 2013) and our studies of IL-6 signaling in aged naïve CD4 T cells that the aged T cells have a higher activation threshold, or as in our studies require a higher dose of inflammatory cytokine, compared to those from the young. Our studies indicate no change in IL-6 receptor expression, but a reduction in downstream effects of receptor-mediated signaling. Whether this reduced

response is caused by changes in ambient IL-6 is unclear, but this seems unlikely since similar response defects are seen in germ-free mice and do not correlate well with IL-6 levels in the serum.

Other defects in T cells may be due partly to physical factors such as changes in cytoskeletal proteins (Garcia and Miller 2011) leading to poor TcR signaling and to increased inhibitory receptors such as PD1, CTLA4 and ICOS (Channappanavar and others 2009). Thus conventional vaccines and adjuvants may fail in part because they do not reach critical levels of TcR and costimulatory receptor triggering during cognate DC:T cell interaction.

6 Increased levels of IL-6 made during DC:T cell interaction enhances aged CD4 T cell responses

Maue et al. found that PolyI:C was an effective adjuvant *in vivo*, and that it increased the helper activity of aged CD4 T cells. However, the pleiotropic effect of adjuvants makes it very difficult to identify their mechanism of action. To study whether we could target effects directly to antigen-specific responding aged T cells, we used TLR agonist-activated DC as APC, which deliver high levels of inflammatory cytokines to cognate antigen-specific T cells with which they interact. Our hope was that this strategy might circumvent aged naïve CD4 defects, resulting in generation of CD4 memory and of enhanced B cell antibody and B cell memory, without the widespread bystander effects caused by systemic adjuvants.

The TLR agonist pre-activated, antigen peptide-pulsed DC, used as APC, made enhanced cytokines when they interacted with specific naïve CD4 T cells, including 40-fold more IL-6, and the naive aged CD4 T cell response *in vitro* was markedly enhanced, so that expansion, survival and IL-2 production, as well as generation of effector and memory cells approached that of young cells. The restoration of the aged CD4 T cell response depended on IL-6 (Jones and others 2010).

Our as yet unpublished studies indicate that the activated DC are also able to initiate a much better response of aged naïve CD4 T cells *in vivo*. The activated DC, acting in an IL-6 dependent mechanism, are able to circumvent most of the age-associated defects at the initiation of response including initial expansion and survival of the aged T cells. To determine if T cell helper function and Tfh were also enhanced, we added formalin-inactivated influenza (A/PR8/34) to provide antigen for B cells, which would also provide a source of B cell APC to drive Tfh.

This resulted in enhanced generation of Tfh and increased IgG antibody to influenza, both of which were IL-6 dependent. Thus the strategy of using pre-activated autologous DC as a way to target the positive effects of adjuvants to specific T cells strongly supports the concept that T cell defects can be overcome by strategies that provide strong stimulus to the cells. Activating the DC enhances the response of young CD4 T cells, but only the aged CD4 T cell response is dependent on IL-6 made by the DC.

These studies define key components responsible for enhancing responses of aged naive CD4 T cells and illustrate the potential of targeted vaccine approaches in the aged that take advantage of the adjuvant properties of TLR ligands.

7 Strategies to target adjuvants to sites of DC:T cell interaction

While our studies so far provide only proof-of-principle for an activated DC-APC based vaccine they take into account the context of aging in which on one hand there is a chronic state of low grade inflammation causing age-related ill effects, while on the other hand there is a need for higher levels of proinflammatory cytokines during antigen presentation to T cells to induce a robust immune response and protection. Using adjuvant treated APC to induce specific T cell responses, would be a safer option for vaccinating the aged, since there is no exposure to systemic adjuvant.

Pioneering studies from Ralph Steinman and Michel Nussenzweig demonstrated the principles of DC-based vaccine, achieved by either *ex vivo* loading of antigen on monocyte derived DCs from patients own monocytes or a more feasible option of *in vivo* targeting using antibodies against DC specific markers (Tacke and others 2007). These strategies have been studied for cancer and chronic infections caused by HIV and HCV (Garcia and others 2013a) and have been extensively reviewed (Garcia and others 2013b; Kreutz and others 2013; Tacke and others 2007). Our studies here point out two key aspects of how such an approach could circumvent T cell aging defects, namely that the DC should be pre-activated to make high levels of IL-6 when they interact with cognate T cells and second, that a source of abundant long-lived antigen for B cells as well as T cells needs to be given. Further studies are planned to determine how well this approach works with aged DC (our preliminary studies indicated these worked well *in vitro*) and in aged mice, where B cells will also have some age-associated defects that could reduce efficacy. Such a DC-targeted approach would be effective and safer for vaccinating aged subjects against influenza and other emerging infections to which the aged are highly susceptible.

We are currently identifying the role of key molecules in the IL-6 pathway and other pathways to define in detail the mechanisms involved, which could identify additional pathways that might be manipulated to enhance aged CD4 T cells response.

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Highlights

- We find impaired T cell help to B cells and a shift in CD4 subsets in the aged.
- Aged CD4 T cells had a dampened response to IL-6
- Adjuvants or addition of IL-6 enhance aged T and B responses.
- Using activated DC as APC can restore poor aged naïve CD4 T cell responses
- This depends on high levels of IL-6 made by DC during cognate interaction.

Fig. 1.

TLR ligand activated antigen pulsed DCs enhance aged naïve CD4 T cell responses. IL-6 plays a crucial role in this function as DCs lacking IL-6 significantly affects the CD4 T cell responses mediated by TLR agonist treated DCs.