Why Aging T Cells Fail: Implications for Vaccination

Commentary

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Summary

The decline in CD4^{+} T cell function with aging contributes to reduced vaccine efficacy. In this commentary, we discuss the factors leading to age-related changes in T cell function and propose how they may be overcome to enhance vaccine efficacy for the elderly.

Introduction

As individuals age, infectious diseases cause increasing morbidity and mortality. In fact, declines in immune function are a hallmark of aging and result in the decreased ability of the elderly to both resist infection and respond to vaccination. This is especially evident when the elderly contract newly emerging diseases such as severe acute respiratory syndrome (SARS), which killed 50% of those over 50 years of age who were infected (Chen et al., 2005). Yearly outbreaks of influenza virus kill 30–50 thousand per year (Thompson et al., 2003) and, if the H5N1 bird flu should become more readily transmissible, the elderly will likely be highly vulnerable.

In light of increasing elderly populations in Western societies, it is critical that we understand what defects in lymphoid cells are responsible for this decline in function, what mechanisms lead to impaired function, and whether poor responses can be improved and/or overcome so that strategies to increase vaccine efficacy can be developed. Several functional defects in lymphoid cells that develop with age have been defined, and they are sufficiently extensive to largely explain the poor vaccination results. However, the cause of these defects and whether they are intrinsic to the cells or an extrinsic consequence of the milieu or history of the individual lymphocytes is unclear.

Defects in Immunization in the Elderly

After a primary viral infection, cell-mediated immunity, including cytotoxic T cell (CTL) functions, are thought to play the largest role in viral clearance (Turner et al., 2004). In contrast, in immunized individuals, protection from infection is most often mediated by virus-specific antibodies (Ab; Thompson et al., 2004). A major problem is that current vaccines exhibit reduced efficacy in the elderly, leading to a high rate of infections in vaccinated individuals. For example, the yearly influenza vaccine has only 40% to 60% efficacy in older individuals (Vu et al., 2002). The elderly also have higher rates of complications such as congestive heart failure and pulmonary disease, resulting in the increased rate of hospitalization and death after influenza infection (Thompson et al., 2004). In addition to influenza, reduced responses in the elderly have been noted with vaccinations for tetanus, encephalitis, hepatitis, and *Streptococcus*

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pneumoniae (Cook et al., 1987; Hainz et al., 2005; Musher et al., 1986), all of which aim to aim to generate a protective antibody response.

The decline in antibody production following vaccination in the elderly is the result of reduced antigen-specific B cell expansion and differentiation, leading to production of reduced titers of antigen-specific IgG (Zheng et al., 1997). Furthermore, the Abs that are produced are of poor quality due to reduced class switching and reduced somatic mutation in the variable region genes resulting in reduced affinity (Song et al., 1997). Thus, Abs produced by older individuals have diminished ability to neutralize and opsonize pathogens, resulting in less protection from subsequent infection.

The generation of high-affinity Ab occurs in germinal centers (GCs), and, importantly, formation of GCs after priming declines with aging (Zheng et al., 1997). The generation of memory B cells, which is also essential for vaccine efficacy, is also highly dependent upon GC (Tsiagbe et al., 1996) and thus, is also reduced with aging. The formation of GC, although not totally understood at this time, is dependent upon CD4^{+} T cell help and their cognate interaction with B cells. In addition to sustained T-B cell contact, cytokine production by CD4^{+} T cells is thought to be essential for robust help (Tsiagbe et al., 1996). Thus, age-related changes in CD4^{+} T cell and/or B cell function are likely to adversely impact the outcome of a humoral response and they may synergize to undermine immunity in the aged.

Defects in T Cells with Aging

One of the most notable changes in the peripheral T cell compartment with aging is the decline in the number of naive T cells in favor of cells with a memory phenotype (Ernst et al., 1990). The ability of naive T cells from aged animals to proliferate and produce cytokines is reduced substantially (Haynes et al., 1999). Studies by Miller and Garcia, using T cell receptor transgenic (TCR Tg) mice, show that CD4^{+} T cells from aged mice do not form immunological synapses as readily as those from younger mice upon stimulation with antigen and antigen presenting cells (Ag-APC; Garcia and Miller, 1997). Furthermore, there is a marked reduction in the recruitment of TCR-associated signaling molecules into the synapse when aged naive CD4^{+} T cells interact with Ag-APC, which is expected to lead to lower levels of initial TCR signals and a reduction in many downstream events.

One functional defect that is of great consequence is cytokine production. Naive CD4^{+} T cells from aged animals produce about half the IL-2 as young cells upon initial stimulation with Ag-APC (Haynes et al., 1999). This defect leads to less clonal expansion and reduced differentiation of effector populations that produce reduced amounts of effector cytokines, with the exception of interferon-γ (IFN-γ). Effectors generated from aged naive CD4^{+} T cells express reduced activation and differentiation markers such as CD25, CD62L, and CD154, suggesting their differentiation is incomplete (Eaton et al., 2004; Haynes et al., 1999). All of these defects are overcome in vitro when IL-2 is provided (Haynes et al., 1999), linking the set of defects to the reduced
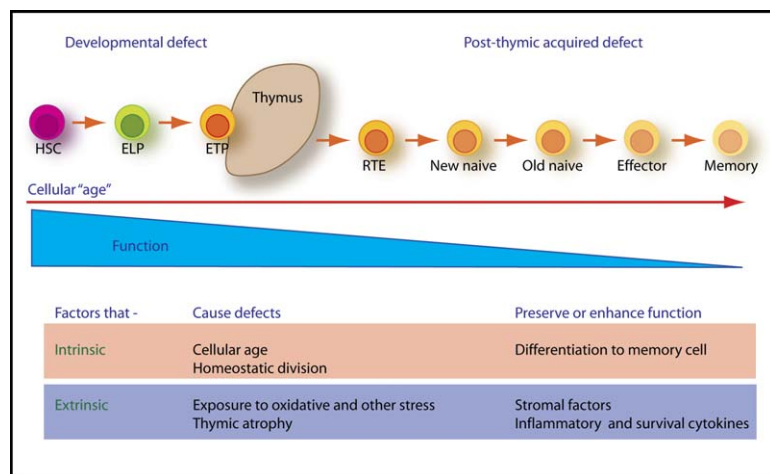
Figure 1. Genesis of Aging Defects

We speculate that as CD4^{+} T cells develop and differentiate, they develop defects in responsiveness and function that accumulate and intensify with time, as indicated. These can be divided into "developmental" defects and "postthymic acquired defects," as shown. Some extrinsic factors, acting at various stages of T cell development, are postulated to have negative effects, including exposure to oxidative and other stress, limitations of survival factors, thymic atrophy, and intrinsic factors such as increased age of the individual cells and their repeated homeostatic division. These are indicated in the red box, and we suggest they combine to undermine functionality. Stromal factors in bone marrow and thymus may act to preserve function. Whereas some defects may be irreversible, others can be reversed or muted by increasing extrinsic positive factors such as survival and inflammatory cytokines, as indicated by the blue box.

production of IL-2 by the aged naive cells. There is also a dramatic decline in the cognate helper activity of CD4^{+} T cells from aged individuals. CD4^{+} T cells from the aged provide little cognate help for induction of humoral responses–leading to reduced B cell expansion, reduced differentiation to GC phenotype and reduced IgG production (Eaton et al., 2004; Zheng et al., 1997).

Memory T cells, generated from aged naive cells stimulated in vitro even in the presence of IL-2, survive and persist well, but they are markedly defective in proliferation and cytokine secretion in recall responses (Haynes et al., 2003). Furthermore, memory cells derived from aged naive CD4^{+} T cells are largely unable to provide cognate help for humoral responses. Surprisingly, IL-2 treatment of defective memory cells cannot restore their defect, suggesting the possibility of epigenetic changes. Paradoxically, although naive CD4^{+} T cells from aged individuals generate memory cells that are largely nonfunctional, memory CD4^{+} T cells generated in young individuals have been shown to retain function for extended periods of time as their host ages. This observation suggests that only T cells at certain stages of development are susceptible to induction of aging defects. Perhaps functional memory needs to be generated in youth or middle age unless strategies to more completely overcome the defects of naive T cells in the elderly can be developed.

Age-related declines in CD4^{+} T cell helper activity may also cause defects in CD4^{+} T cell-dependent aspects of CD8^{+} T cell responses. CD4^{+} T cells are critical for establishment and/or maintenance of the CD8^{+} T cell memory pool probably via CD154 (Schoenberger et al., 1998). CD8^{+} memory T cells generated without appropriate CD4^{+} T cell help are defective in recall responses and undergo activation-induced cell death upon reencounter with antigen. Naive CD4^{+} T cells from aged animals express less CD154 than young cells (Eaton et al., 2004), and this may lead to the generation of fewer and less enduring CD8^{+} T memory cells with greatly reduced function, although this premise has not yet been directly tested. We suggest that loss of naive CD4^{+} T cell function and consequent poor generation of Ab-producing B cells, coupled with poor CD8^{+} T cell priming, synergize

to produce poor T and B cell effector function after primary immunization and poorly functional memory cells of all types. This may explain both the inadequate responses of elderly patients to new pathogens they have not encountered earlier in their lives as well as their failure to be adequately vaccinated.

Genesis of T Cell Defects

Defects in naive CD4^{+} T cells seem to occur at multiple levels. Production of new naive T cells declines dramatically with age. This is probably due to intrinsic defects in the stem cells or in the thymus itself (Min et al., 2004). Because the size of the total peripheral T cell compartment changes little, it is likely that the postthymic life span of T cells must increase to maintain T cell numbers. We have proposed that this longer residence of T cells in the periphery with age contributes to the reduced function (Haynes et al., 2005). Because persisting naive T cells acquire this defect over time in the periphery, we have termed this the "postthymic acquired defect" (Figure 1). It is easy to imagine that a series of factors could work to erode T cell function over time. With aging, persisting naive T cells, which divide slowly, if at all (Tough and Sprent, 1994), will be exposed to extrinsic factors such as oxidative stress, possible limitations in availability of survival factors, and other unidentified environmental factors. These factors may induce programs in the T cells that favor persistence at the expense of future function. To the extent the T cells do turnover, homeostatic turnover itself may also limit their potential (Swain et al., 2005).

Our studies support the idea that persistence in the periphery leads to a progressive decrease in function. New CD4^{+} T cells generated in aged animals from aged stem cells function well in terms of proliferation and IL-2 production (Haynes et al., 2005). Similar findings are seen when CD4^{+} T cells are depleted in young and aged TCR Tg mice by in vivo Ab treatment (Haynes et al., 2005). After repopulation, new CD4^{+} T cells generated in aged mice proliferated and made IL-2 as well as T cells from young animals. Equivalent results are found when new polyclonal populations are generated in young and aged nontransgenic mice. Newly generated CD4^{+} T cells exhibit robust cognate function, leading

to enhanced humoral responses, again suggesting that a portion of the aging defect is a result of postthymic acquired defects.

Further support for this hypothesis of intensifying defects acquired as T cells age comes from studies in which the aging of the peripheral T cell population is accelerated by thymectomy of young TCR Tg mice. The cohort of T cells recovered from thymectomized mice become less responsive starting at 8 months instead of 14 months in sham thymectomized controls (L.H. and S.L.S., unpublished data). Thus, halting production of new T cells leads to reduced ex vivo response of the remaining cells to Ag-APC. This defect could be due to several factors, but it is compatible with the concept that postthymic persistence leads to defects that accumulate with time.

It is also possible that the renewed function of naive T cells seen in the reconstitution models is in some part due to the conditions under which they developed. Indeed, defects in reconstitution potential are already present in stem cells of aged mice, and these also are expected because they too have been endured a longer exposure to environmental stimuli. The frequency and proliferation of early T lineage precursors (ETP) declines with age (Min et al., 2004), leading to reduced pre-T cell production. To evaluate the role of defects acquired during development, we have examined whether T cells at earlier stages of development exhibit some of the defects seen later. We find that recent thymic emigrants (RTE), which are likely to be derived largely from ETP, exhibit age-related defects in proliferation and IL-2 production, which we have termed the "developmental defect" (K. Clise-Dwyer and S.L.S., unpublished data).

Thus, we propose that defects in CD4$^+$ T cell function with aging are the consequence of multiple defects that occur at different stages of the T cell life span. One set of defects (the developmental defect) occurs early before the generation of RTE and another (the postthymic acquired defect) occurs much later, after a lengthy stay in the peripheral environment. This accumulation of defects at several stages of the CD4$^+$ T cell life span, as a result of multiple extrinsic and intrinsic factors, is illustrated in Figure 1. Importantly, at least some of the functional defects can be overcome by a variety of factors discussed below.

Overcoming Aging Defects: Promise of Adjuvants

Importantly, extrinsic factors may be able to enhance aged T cell function or reverse defects, suggesting that some aging defects are either reversible or that their impact could be muted by adding such factors (Haynes et al., 1999, 2004). Both survival factors such as IL-2 and inflammatory cytokines can markedly augment responses of aged naive cells both in vitro and in vivo.

One of the most clinically important age-related changes in immune function is the decline in efficacy of vaccinations. A straightforward approach to enhancing vaccine efficacy is to employ more potent adjuvants. Adjuvants work, in part, by improving the activation of APCs. APCs express toll-like receptors (TLR), which have the ability to sense pathogen-associated molecular patterns from various kinds of pathogens (bacterial, viral, parasitic; Iwasaki and Medzhitov, 2004). One of the most promising TLR binding adjuvants is prokaryotic unmethylated CpG containing oligodeoxynucleo-

tide (CpG-ODN; Klinman, 2004). Studies have shown that using CpG-ODN as an adjuvant can enhance the response to vaccination in aged animals and boost Ab production (Maletto et al., 2005). Many TLR binding adjuvants also induce APC to produce inflammatory cytokines such as TNFα, IL-1, and IL-6 (Iwasaki and Medzhitov, 2004), which can also enhance the function of naive CD4$^+$ T cells from aged animals. This enhanced function includes improved clonal expansion and IL-2 production (Haynes et al., 2004) and also enhanced cognate helper function, leading to increased Ab production (L.H., S. Eaton, and A. Maue, unpublished data). Thus, residual functions of "defective" aged naive CD4$^+$ T cells can be improved, leading to enhanced vaccine efficacy. When the pathways involved in enhancement of function are defined, it should be possible to use TLR agonists that stimulate particular pathways or other agonists of pattern recognition receptors to make vaccines customized for the elderly.

It is also important to note that we believe that enhancing aged CD4$^+$ T cell function alone will lead to improved Ab production. Young CD4$^+$ T cells transferred into immunized aged hosts function well, resulting in robust humoral responses (Eaton et al., 2004). Thus, other components of the humoral immune response, such as B cells and follicular dendritic cells, do not lose function with aging as dramatically as T cells. In addition, APCs, such as dendritic cells, also do not seem to decline in function with aging. Dendritic cells from young and aged mice produce similar levels of inflammatory cytokines in response to TLR ligands (R. Larson and L.H., unpublished data), and CpG-stimulated dendritic cells can dramatically boost the in vitro responses of aged CD4$^+$ T cells (S. Jones and S.L.S., unpublished data). In the future, it will be interesting to determine if there is a direct link between the observation that inflammatory cytokines overcome aging defects in CD4$^+$ T cell function during an immune response and that generation of new CD4$^+$ T cells in a highly lymphopenic, possibly inflamed, environment (see Figure 1) overcomes some of the defects in RTE developing in intact aged mice.

Questions for the Future

Some aspects of aging are readily explained by known factors that result in decreased numbers of peripheral naive lymphocytes, such as decreased stem cell activity and decreased thymic output. However, we have little understanding of why aged naive lymphoid cells fail or explanations why some cells, like memory cells, seem to escape the impact of prolonged survival. We also do not understand what molecular changes in the lymphocytes are responsible for their impaired function. If we can begin to understand the factors that induce aging defects and in turn define what these defects are, it may be possible to design multiple new strategies to compensate for the defects. It is especially important to thoroughly evaluate how well and for how long adjuvants can improve the responses of aged T cells.

References

Chen, Q., Liang, W.N., Liu, G.F., Liu, M., Xie, X.Q., Wu, J., He, X., and Liu, Z.J. (2005). Case fatality rate of severe acute respiratory syndromes in Beijing. Biomed. Environ. Sci. *18*, 220–226.

Cook, J.M., Gualde, N., Hessel, L., Mounier, M., Michel, J.P., Denis, F., and Ratinaud, M.H. (1987). Alterations in the human immune response to the hepatitis B vaccine among the elderly. Cell. Immunol. *109*, 89–96.

Eaton, S.M., Burns, E.M., Kusser, K., Randall, T.D., and Haynes, L. (2004). Age-related defects in CD4 T cell cognate helper function lead to reductions in humoral responses. J. Exp. Med. *200*, 1613–1622.

Ernst, D.N., Hobbs, M.V., Torbett, B.E., Glasebrook, A.L., Rehse, M.A., Bottomly, K., Hayakawa, K., Hardy, R.R., and Weigle, W.O. (1990). Differences in the expression profiles of CD45RB, Pgp-1 and 3G11 membrane antigens and the pattern of lymphokine secretion by splenic CD4+ T cells from young and aged mice. J. Immunol. *145*, 1295–1302.

Garcia, G.G., and Miller, R.A. (1997). Differential tyrosine phosphorylation of zeta chain dimers in mouse CD4 T lymphocytes: effect of age. Cell. Immunol. *175*, 51–57.

Hainz, U., Jenewein, B., Asch, E., Pfeiffer, K.P., Berger, P., and Grubeck-Loebenstein, B. (2005). Insufficient protection for healthy elderly adults by tetanus and TBE vaccines. Vaccine *23*, 3232–3235.

Haynes, L., Linton, P.-J., Eaton, S.M., Tonkonogy, S.L., and Swain, S.L. (1999). IL-2, but not other common γ chain (γc)-binding cytokines, can reverse the defect in generation of CD4 effector T cells from naive T cells of aged mice. J. Exp. Med. *190*, 1013–1023.

Haynes, L., Eaton, S.M., Burns, E.M., Randall, T.D., and Swain, S.L. (2003). CD4 T cell memory derived from young naive cells functions well into old age, but memory generated from aged naive cells functions poorly. Proc. Natl. Acad. Sci. USA *100*, 15053–15058.

Haynes, L., Eaton, S.M., Burns, E.M., Rincon, M., and Swain, S.L. (2004). Inflammatory cytokines overcome age-related defects in CD4 T cell responses in vivo. J. Immunol. *172*, 5194–5199.

Haynes, L., Eaton, S.M., Burns, E.M., Randall, T.D., and Swain, S.L. (2005). Newly generated CD4 T cells in aged animals do not exhibit age-related defects in response to antigen. J. Exp. Med. *201*, 845–851.

Iwasaki, A., and Medzhitov, R. (2004). Toll-like receptor control of the adaptive immune responses. Nat. Immunol. *5*, 987–995.

Klinman, D.M. (2004). Immunotherapeutic uses of CpG oligodeoxynucleotides. Nat. Rev. Immunol. *4*, 249–258.

Maletto, B.A., Ropolo, A.S., Liscovsky, M.V., Alignani, D.O., Glocker, M., and Pistoresi-Palencia, M.C. (2005). CpG oligodeoxinucleotides functions as an effective adjuvant in aged BALB/c mice. Clin. Immunol. *117*, 251–261.

Min, H., Montecino-Rodriguez, E., and Dorshkind, K. (2004). Reduction in the developmental potential of intrathymic T cell progenitors with age. J. Immunol. *173*, 245–250.

Musher, D.M., Chapman, A.J., Goree, A., Jonsson, S., Briles, D., and Baughn, R.E. (1986). Natural and vaccine-related immunity to *Streptococcus pneumoniae*. J. Infect. Dis. *154*, 245–256.

Schoenberger, S.P., Toes, R.E., van der Voort, E.I., Offringa, R., and Melief, C.J. (1998). T-cell help for cytotoxic T lymphocytes is mediated by CD40–CD40L interactions. Nature *393*, 480–483.

Song, H., Price, P.W., and Cerny, J. (1997). Age-related changes in antibody repertoire: contribution from T cells. Immunol. Rev. *160*, 55–62.

Swain, S., Clise-Dwyer, K., and Haynes, L. (2005). Homeostasis and the age-associated defect of CD4 T cells. Semin. Immunol. *17*, 370–377.

Thompson, W.W., Shay, D.K., Weintraub, E., Brammer, L., Cox, N., Anderson, L.J., and Fukuda, K. (2003). Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA *289*, 179–186.

Thompson, W.W., Shay, D.K., Weintraub, E., Brammer, L., Bridges, C.B., Cox, N.J., and Fukuda, K. (2004). Influenza-associated hospitalizations in the United States. JAMA *292*, 1333–1340.

Tough, D.F., and Sprent, J. (1994). Turnover of naive- and memory-phenotype T cells. J. Exp. Med. *179*, 1127–1135.

Tsiagbe, V.K., Inghirami, G., and Thorbecke, G.J. (1996). The physiology of germinal centers. Crit. Rev. Immunol. *16*, 381–421.

Turner, S.J., Kedzierska, K., La Gruta, N.L., Webby, R., and Doherty, P.C. (2004). Characterization of CD8+ T cell repertoire diversity and persistence in the influenza A virus model of localized, transient infection. Semin. Immunol. *16*, 179–184.

Vu, T., Farish, S., Jenkins, M., and Kelly, H. (2002). A meta-analysis of effectiveness of influenza vaccine in persons aged 65 years and over living in the community. Vaccine *20*, 1831–1836.

Zheng, B., Han, S., Takahashi, Y., and Kelsoe, G. (1997). Immunosenescence and germinal center reaction. Immunol. Rev. *160*, 63–77.