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Gene expression and generation of CD28⁻ CD8 T cells mediated by interleukin 15

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Abstract

Accumulation of CD28⁻CD8 T cells that are defective in response to antigenic stimulation is a hallmark of age-associated decline in T cell function. However, the underlying mechanism of this age-associated change is not fully understood. We recently analyzed the global gene expression profiles of CD8 T cell subsets from naïve to memory (CD28⁺ to CD28⁻) cells and the growth of CD28⁺ and CD28⁻ CD8 memory T cells in response to homeostatic cytokine interleukin 15 (IL-15). At the gene expression level, one of the most striking changes is the altered expression of some co-stimulatory receptors and various NK cell receptors in CD28⁻ CD8 T cells. Furthermore, CD28⁻ CD8 T cells appear to have a normal proliferation response to IL-15 in vitro. Interestingly, IL-15 is also capable of inducing stable loss of CD28 expression in actively dividing CD28⁺CD8 memory T cells. Together, these findings provide the gene expression features of CD28⁻ CD8 T cells that differ from their CD28⁺ counterparts and suggest a possible role of IL-15 in the increase of CD28⁻CD8 T cells that occurs with aging.

Keywords

Aging; NK cell receptor; co-stimulatory receptor

Introduction

CD8 T cells are an essential part of the adaptive immune system against intracellular pathogens and cancerous growths. With age, the ability of CD8 T cells to protect the host deteriorates, which in combination with other age-associated changes, leads to the decline of overall immune function (Weng, 2006). Although the mechanism of age-associated decline of immune function is not fully understood, accumulation of CD28⁻CD8 T cells that are defective in antigen-induced proliferation and have restricted clonal diversity is consistently observed in the elderly and is at least partially responsible for this decline of immune function (Azuma, et al., 1993; Fagnoni, et al., 1996).

The effectiveness of CD8 T cell function relies on their response to antigen via T cell receptor (TCR) and co-stimulatory receptors such as CD28. Because CD28 provides a pivotal role during T cell activation, such as inducing cytokine production (IL-2) and promoting cell proliferation (Acuto and Michel, 2003), lack of this co-stimulatory signal during activation results in a partial activation or even an anergy state of T cells. At birth, T cells in peripheral

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blood are naïve cells expressing CD28 on their surfaces. In adulthood, loss of CD28 expression is found predominantly in antigen-experienced memory T cells, particularly in CD8 T cells, and the number of those CD28⁻ CD8 T cells increases significantly with increasing age. CD28⁻ CD8 T cells constitute up to 60-80% of total CD8 T cells in individuals over 80 years old (Fagnoni, et al., 1996). The cause for loss of CD28 expression in T cells and their subsequent accumulation in the elderly are not completely understood. Increasing evidence suggest that chronic stimulation of T cells by certain viruses is a major cause of loss of CD28 expression in CD8 T cells (Effros, et al., 1996).

Profound changes have been observed in those CD28⁻ CD8 T cells such as (1) reduced proliferative response to stimulation (Posnett, et al., 1994); (2) defective cytokine production such as IL-2 and IFN γ in response to stimulation (Nociari, et al., 1999); (3) resistance to apoptosis (Spaulding, et al., 1999); (4) reduced antigen repertoire diversity (Posnett, et al., 1994), and (5) associated with the lack of antibody production after immunization (Saurwein-Teissl, et al., 2002). In addition, CD28⁻ CD8 T cells gain expression of some NK cell markers such as KIRs, CD16, CD56, KLRK1 (NKG2D) and retain or increase cytotoxicity with high expression of granzyme B and perforin (Azuma, et al., 1993).

Despite recent findings, the key issues related to CD28⁻ CD8 T cells are still unclear. What are the mechanisms that cause an irreversible loss of CD28 expression in T cells? What are the molecular alterations of CD28⁻CD8 T cells compared to CD28⁺CD8 naïve and memory T cells? How are CD28⁻ CD8 memory T cells maintained in vivo? Here, we summarize our recent findings on 1) gene expression changes involved in co-stimulatory and NK receptors during the differentiation of CD28⁺ naïve and memory CD8 T cells to CD28⁻ memory and effector memory cells and 2) growth and generation of CD28⁻ CD8 memory T cells under the homeostatic cytokine IL-15.

Altered expression of co-stimulatory receptors in CD28⁻ CD8 T cells

Recently, we have analyzed global gene expression profiles of different subsets of CD8 T cells from naïve to CD28⁺ and CD28⁻ memory cells (Fann, et al., 2005; Fann, et al., 2006). These analyses lead to the identification of a number of genes that are differentially expressed in naïve CD8 T cells and memory CD8 T cell subsets. Here we focus our discussion on the changes of expression of co-stimulatory and NK cells receptors in CD28⁻ CD45RA⁺ memory CD8 T cells and with additional data on the CD45RA⁺CD28⁻ effector memory CD8 T cells.

The CD28 co-stimulatory receptor family consists of a single extracellular IgV domain and a cytoplasmic tail and has five known members including CD28, CTLA-4 (CD152), inducible costimulator (ICOS), program death-1 (PD-1, PDCD-1), and B and T lymphocyte attenuator (BTLA) (Riley and June, 2005). The expression levels of three members of the CD28 family are changed during CD8 T cell differentiation from naïve to memory (CD28⁺ to CD28⁻) to effector memory CD8 T cells (Fig. 1A). The level of CD28 is highest in naïve cells and gradually decreases from memory (CD28⁺ to CD28⁻) to effector memory cells. The expression of ICOS is similar between naïve and memory (CD28⁺) but is dramatically down-regulated in both CD28⁻ memory and effector memory CD8 T cells. In contrast, the highest level of CTLA4 is found in memory (CD28⁺) cells and the lowest level in CD28⁻ effector memory CD8 T cells.

CD40L and CD70 are members of the TNF superfamily and both serve as co-stimulatory receptors during T cell activation. The expression pattern of CD40L is similar to CTLA4 during CD8 T cell differentiation (Fig. 1A). CD70 is a ligand for CD27, a receptor that is member of the tumor necrosis factor receptor (TNFR family). The signal generated from CD27/CD70 interaction is temporally or spatially segregated from CD28 during T cell activation (Watts, 2005). The T cell activation/survival signals generated by different co-stimulators have some functions in common and yet distinct from each other in other aspects to allow effectiveness

and longevity of the T cell response and survival. The levels of CD27 and CD70 are stable from naive to memory (CD28⁺ to CD28⁻) cells. Decreased CD27 expression associated with increased CD70 expression are found in the effector memory CD8 T cells (Fig. 1A). The significance of this altered balance of CD27/CD70 expression remains to be determined. It is clear, however, that the parallel loss of CD27 and CD28 expression has profound impact on CD8 T cell function.

Altered expression of NK cell receptors in CD28⁻CD8 T cells

Based on their structures, NK cell receptors can be divided into 1) the Immunoglobulin-like NK cell receptors including natural cytotoxicity receptors (NCR), killer immunoglobulin-like receptor (KIR), and CD244, and 2) the C-type lectin-like NK cell receptors including the killer cell lectin-like receptor (KLR) (Bianconi, et al., 2003). Based on their functions, NK cell receptors can be divided into inhibitory and stimulatory receptors. While engagement of the inhibitory receptors prevents NK cells and CD8 T cells from killing target cells, interaction of stimulatory receptors results in the triggering of NK cell or CD8 T cell-mediated cytotoxicity. Expression of NK cell receptors on CD28⁻ CD8 T cells have been reported (Phillips, et al., 1995). Here we discuss the expression of seven NK cell receptors: KIR2DL2, KIR3DL2, NCR1, CD244, KLRD1, KLRF1, and KLRG1 during CD8 T cell differentiation (Fig. 1B).

KIR2DL2, KIR3DL2, NCR1, and CD244 belong to the Ig-like NK cell receptor family. KIR2DL2 (NKAT6) and KIR3DL2 (NKAT4) bind to the polymorphic MHC class I molecules and inhibits lymphocyte cytotoxicity. In contrast, NCR1 and CD244 are stimulatory NK receptors that activate NK-mediated cytotoxicity. Despite their opposite function, the expression patterns of these four NK cell receptors are similar: all of them are expressed in naïve cells, down-regulated in CD28⁺ memory cells, increased in CD28⁻ memory cells, and elevated in effector memory cells (Fig. 1B). After IL-15 treatment, all of them are down-regulated in CD28⁻CD8 memory T cells (Fann, et al., 2005). As NCR1 is considered to be exclusively expressed in NK cells, the role of its elevated expression in effector memory T cells remains to be determined. It has been shown that CD244 level is elevated in cytomegalovirus (CMV)-specific effector CD8 T cells while absent in naïve CD8 T cells (Speiser, et al., 2001). This enhanced expression of CD244 in effector memory cells agrees with the elevated effector function of the CMV specific CD8 T cells.

KLRD1, KLRF1, and KLRG1 belong to the C-type lectin-like NK cell receptor family. KLRD1 (CD94) forms heterodimers with KLRC3 (NKG2E) or other members. The KLRD1/KLRK1 (CD94/NKG2D) heterodimer is expressed primarily in NK cells and CD8 T cells. KLRF1 (Nkp80) is expressed in all NK cells and CD56⁺ T cells and cross-linking of KLRF1 results in induction of cytolytic activity. KLRG1 is a newly identified member of the KLR family and is expressed in NK cells and a subset of T cells. Although all of them are highly expressed in CD28⁻ CD8 T cells, the patterns of their expression differ. A down-regulation of expression from naïve to memory (CD28⁺) is observed for KLRD1 and KLRF1 but not for KLRG1 (Fig. 1B). The identification of elevated expression of different NK cell receptors in CD28⁻ CD8 memory T cells suggests that NK cell receptors may play roles in CD28⁻ CD8 T cell function. The physiological significance of acquiring these NK cell receptors in CD28⁻ CD8 T cells will require further study.

Growth and generation of CD28⁻ CD8 T cells in vitro by IL-15

It appears paradoxical that CD28⁻ CD8 T cells are defective in antigen-induced proliferation and yet their numbers increase with age. As memory CD8 T cells are maintained by homeostatic cytokines, we compared the proliferation rate of CD28⁻ CD8 memory T cells to homeostatic cytokine IL-15 with their CD28⁺ counterparts. Our study shows that the proliferation rates are similar between CD28⁺ and CD28⁻ CD8 memory T cells in response to IL-15 in vitro when

measured by a cell division tracking dye (CFSE) (Chiu, et al., 2006) (Fig. 2A). We then analyzed if age influences IL-15-induced cell division of CD28⁺CD8 memory T cells by comparing CD8 T cells isolated from young (mean age 23 years old) and old (mean age 82 years old) donors in response to IL-15 in vitro. We found that CD28⁺ and CD28⁺CD8 memory T cells from both age groups had similar proliferation rates (Fig. 2A). Our findings suggest that CD28⁺ CD8 memory T cells have no defects in IL-15 induced proliferation and that age does not affect the proliferation rates of CD28⁺CD8 T cells in response to IL-15.

In addition to antigen-stimulation-induced loss of CD28 expression, IL-15 is also shown to be capable of down-regulating CD28 expression in CD28⁺CD8 T cells (Borthwick, et al., 2000). However, it is unknown whether IL-15-mediated loss of CD28 expression in CD8 T cells is transient or stable. We have recently analyzed the kinetic expression of CD28 on CD28⁺ CD8 memory T cells cultured with IL-15 (Chiu, et al., 2006). We found that CD28 expression was stable during the initial three to four cell divisions but sustained a rapid loss of CD28 expression in cells that undergo more than five cell divisions (Fig. 2B). The IL-15-mediated loss of CD28 expression in CD8 memory T cells is at the transcriptional level and such a loss is stable in culture. We further demonstrated that CD28⁺ cells generated after IL-15 treatment were defective at inducing IL-2 and IL-2 receptor alpha (IL-2R α or CD25) expression after in vitro activation with anti-CD3 plus anti-CD28 antibodies. Together, these findings suggest that IL-15-mediated down-regulation of CD28 expression occurs mainly in actively dividing CD28⁺CD8 memory T cells and that IL-15-induced loss of CD28 expression in CD8 memory T cells is stable under continuous IL-15 stimulation. Thus, homeostatic cytokine IL-15-mediated growth of CD8 memory T cells not only maintains CD28⁺ CD8 memory T cells but also generates CD28⁺ CD8 memory T cells from their CD28⁺ counterparts. More studies will be needed to understand the role of homeostatic cytokines in CD8 T cell maintenance and aging.

Conclusion

Global gene expression provides a powerful tool to identify age-associated transcriptional changes in CD8 T cells. Here we show that loss of CD28 expression in CD8 T cells during aging is accompanied by an array of altered expressions of co-stimulatory and NK cell receptors. However, the functional significance of the changing expression of these genes in CD8 T cell aging requires further study. In addition, we discuss the growth and cause of CD28⁺CD8 T cells in aging and provide evidence that homeostatic cytokine IL-15 can contribute to the generation and accumulation of CD28⁺CD8 T cells. Further characterization of the regulation and function of those differentially expressed genes in CD28⁺ CD8 T cells will help us to better understand this age-associated change in T cell function.

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References

- Acuto O, Michel F. CD28-mediated co-stimulation: a quantitative support for TCR signalling. *Nat Rev Immunol* 2003;3:939–951. [PubMed: 14647476]
- Azuma M, Phillips JH, Lanier LL. CD28-T lymphocytes: antigenic and functional properties. *J Immunol* Feb 15;1993 150:1147–1159. [PubMed: 8381831]
- Biassoni R, Cantoni C, Marras D, Giron-Michel J, Falco M, Moretta L, Dimasi N. Human natural killer cell receptors: insights into their molecular function and structure. *J Cell Mol Med* 2003;7:376–387. [PubMed: 14754506]

- Borthwick NJ, Lowdell M, Salmon M, Akbar AN. Loss of CD28 expression on CD8(+) T cells is induced by IL-2 receptor gamma chain signalling cytokines and type I IFN, and increases susceptibility to activation-induced apoptosis. *Int Immunol* 2000;12:1005–1013. [PubMed: 10882412]
- Chiu WK, Fann M, Weng NP. Generation and growth of CD28^{null}CD8⁺ memory T cells mediated by IL-15 and its induced cytokines. *J Immunol*. 2006in press
- Effros RB, Allsopp R, Chiu CP, Hausner MA, Hirji K, Wang L, Harley CB, Villeponteau B, West MD, Giorgi JV. Shortened telomeres in the expanded CD28-CD8+ cell subset in HIV disease implicate replicative senescence in HIV pathogenesis. *AIDS* 1996;10:F17–F22. [PubMed: 8828735]
- Fagnoni FF, Vescovini R, Mazzola M, Bologna G, Nigro E, Lavagetto G, Franceschi C, Passeri M, Sansoni P. Expansion of cytotoxic CD8+CD28- T cells in healthy ageing people, including centenarians. *Immunology* 1996;88:501–507. [PubMed: 8881749]
- Fann M, Chiu WK, Wood WH III, Levine BL, Becker KG, Weng NP. Gene expression characteristics of CD28null memory phenotype CD8+ T cells and its implication in T-cell aging. *Immunol Rev* 2005;205:190–206. [PubMed: 15882354]
- Fann M, Godlove JM, Catalfamo M, Wood WH Iii, Chrest FJ, Chun N, Granger L, Wersto R, Madara K, Becker K, Henkart PA, Weng NP. Histone acetylation is associated with differential gene expression in the rapid and robust memory CD8+ T cell response. *Blood* 2006;108:3363–3370. [PubMed: 16868257]
- Nociari MM, Telford W, Russo C. Postthymic development of CD28-CD8+ T cell subset: age-associated expansion and shift from memory to naive phenotype. *J Immunol* Mar 15;1999 162:3327–3335. [PubMed: 10092786]
- Phillips JH, Gumperz JE, Parham P, Lanier LL. Superantigen-dependent, cell-mediated cytotoxicity inhibited by MHC class I receptors on T lymphocytes. *Science* May 21;1995 268:403–405. [PubMed: 7716542]
- Posnett DN, Sinha R, Kabak S, Russo C. Clonal populations of T cells in normal elderly humans: the T cell equivalent to “benign monoclonal gammopathy”. *J Exp Med* Feb 1;1994 179:609–618. [PubMed: 8294871]
- Riley JL, June CH. The CD28 Family: a T Cell Rheostat for Therapeutic Control of T Cell Activation. *Blood* Jan 1;2005 105:13–21. [PubMed: 15353480]
- Saurwein-Teissl M, Lung TL, Marx F, Gschosser C, Asch E, Blasko I, Parson W, Bock G, Schonitzer D, Trannoy E, Grubeck-Loebenstien B. Lack of antibody production following immunization in old age: association with CD8(+)CD28(-) T cell clonal expansions and an imbalance in the production of Th1 and Th2 cytokines. *J Immunol* Jan 6;2002 168:5893–5899. [PubMed: 12023394]
- Spaulding C, Guo W, Effros RB. Resistance to apoptosis in human CD8+ T cells that reach replicative senescence after multiple rounds of antigen-specific proliferation. *Exp Gerontol* 1999;34:633–644. [PubMed: 10530789]
- Speiser DE, Colonna M, Ayyoub M, Cella M, Pittet MJ, Batard P, Valmori D, Guillaume P, Lienard D, Cerottini JC, Romero P. The activatory receptor 2B4 is expressed in vivo by human CD8+ effector alpha beta T cells. *J Immunol* 2001;167:6165–6170. [PubMed: 11714776]
- Watts TH. TNF/TNFR family members in costimulation of T cell responses. *Annu Rev Immunol* 2005;23:23–68. [PubMed: 15771565]
- Weng NP. Aging of the immune system: how much can the adaptive immune system adapt? *Immunity* 2006;24:495–499. [PubMed: 16713964]

Fig 1. Altered expression of co-stimulatory and NK cell receptors in CD28⁻ CD8 T cells. A. Co-stimulatory receptor expression in CD8 T cell subsets. B. NK cell receptor expression in CD8 T cell subsets. The expression level is relative to the level of β -actin.

Fig 2.

Growth and generation CD28⁻ CD8 T cells under IL-15 in vitro. A. IL-15-induced proliferation of CD28⁺ and CD28⁻ memory CD8 T cells from young and old donors (n=4). B. Loss of CD28 expression in frequently dividing CD28⁺ memory CD8 T cells in IL-15 culture. Freshly isolated CD28⁺ CD8 memory T cells were labeled with CFSE and cultured in the presence of IL-15. At day 14 of culture, cells were harvested for analyses of cell divisions determined by CFSE dilution and the percentage of CD28⁺ and CD28⁻ CD8 memory T cells at each division by FACS. The mean values of the percentage of CD28⁺ and CD28⁻ CD8 memory T cells at each division after 14 day culture from 11 donors are presented.