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Enhancement of Virus-Specific Expansion of Transgenic CD8 T Cells in Aged Mice by Dendritic Cells

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Abstract

Aging is associated with a decreased CD8 T cell response to multiple antigens and to virus infection. Although both intrinsic and extrinsic factors have been shown to contribute to the decrease, the mechanisms are still largely unknown. In this study, the role of dendritic cells (DCs) in the age-associated decrease was examined. Influenza-specific TCR transgenic CD8 T cells of young mice demonstrated limited expansion in response to influenza infection when adoptively transferred to aged compared to young mice. This decreased response in aged mice could be significantly enhanced when DCs of young mice were co-transferred. Co-transfer of DCs had no impact in young recipient mice. Adoptive transfer of the DCs also increased the endogenous CD8 T cell response of intact aged mice, although to a lesser degree. These results suggest that the diminished CD8 T cell response to virus infection in aged mice is partially attributable to age-associated changes in DCs.

Keywords

T Cells; Viral; Dendritic Cells; Aging

1. Introduction

Aging is associated with a decreased CD8 T cell response to virus infection (Po et al., 2002; Kapasi et al., 2002; Jiang et al., 2009; Brien et al., 2009), although the mechanisms are still largely unclear (Linton et al., 2005). Using an adoptive transfer approach with two different viruses, we observed that the aged environment, regardless of genetic background of mice, significantly inhibits both clonal expansion and IFN- γ production by specific Tg CD8 T cells of young mice during virus infection (Jiang et al., 2009). These data indicate that alterations in the aged environment play an important role in the decreased specific CD8 T cell immunity to virus infection with aging.

Dendritic cells (DCs) are one component of the lymphoid environment that could contribute to the diminished response with aging. Several studies have described a reduced ability of unfractionated APCs or macrophages of aged mice to stimulate T cell effector function (Jiang et al., 2009; Plowden et al., 2004; Donnini et al., 2002; Beharka et al., 1997). Poor expression

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of co-stimulatory molecules, such as CD40 and CD86, on DCs of aged mice may result in failure to induce optimal effector T cell responses (Chiu et al., 2007; Varas et al., 2003). Recently, Grolleau-Julius et al (Grolleau-Julius et al., 2008) observed a decrease in DC-SIGN (CD209) expression in aged mice, which may contribute to impaired T cell response. In addition, adoptive transfer experiments show defective trafficking of DCs *in vivo* in aged mice (Linton et al., 2005; Grolleau-Julius et al., 2008). While some studies report a decline in function of mouse DCs (Sprecher et al., 1990; Villadsen et al., 1987; Haruna et al., 1995), other studies report no change (Tesar et al., 2006; Komatsubara et al., 1986). Similarly, no consensus has been reached concerning the function of DCs of humans (Lung et al., 2000; Shurin et al., 2007; Saurwein-Teissl et al., 1998). Our recent data (Jiang et al., 2009) suggest that the *in vitro* function of DCs remain intact with age: when equal numbers of purified DCs from young or aged mice were cultured with the same virus-specific CD8 T cells of young mice, the expansion of the CD8 T cells was similar, indicating that the APC function of DCs from aged mice remains intact *in vitro*. These results supported a previous study that showed the functions of DCs from aged mice remain intact even though there exists differences in the percentages of myeloid vs lymphoid DCs and surface expression of MHC molecules on DCs between young and aged mice (Norian and Allen, 2004).

It has been reported that the number of DCs recovered from spleens of aged mice is decreased 30–50% compared to young mice (Linton et al., 2005). Our recent data also show that both BALB/c and B6 mice demonstrate a significantly decreased number of DCs in the spleen of aged compared to young mice (Jiang et al., 2009). This decreased number of DCs in aged mice may contribute to the inability of the aged environment to support maximal expansion of CD8 T cells in aged mice.

The current study was designed to determine if the number of DCs in aged mice was limiting T cell expansion. Utilizing Tg CD8 T cells specific for influenza as a model, we found that the diminished expansion of the Tg CD8 T cells that is observed in aged compared to young recipients after influenza virus infection was significantly enhanced in aged mice when DCs of young mice were co-transferred. A similar effect was not observed in young recipients. Interestingly, adoptive transfer of enriched DCs failed to significantly enhance the endogenous CD8 T cell response in aged mice. These results suggest that DCs in the aged environment are not sufficient to support the expansion of young CD8 T cells in response to antigen-specific challenge in this model influenza virus infection. However, since the diminished endogenous CD8 T cell response with aging cannot be enhanced by adoptively transferred DCs, either the number of DCs transferred needs to be larger, the transferred DCs do not migrate adequately, or the DCs cannot compensate for the intrinsic defect of CD8 T cells of aged mice.

2. Materials and Methods

2.1. Mice, virus, and infection

Young (4-month-old) and aged (18–20-month-old) female wild type Thy1.2⁺ BALB/c mice were purchased from the NIA at Harlan Sprague Dawley (Indianapolis, IN). Six-to 8-week old female Thy1.1⁺ BALB/c ByJ Cl.1, Clone-4 (HA_{518–526} TCR-Tg) mice, specific for influenza virus (Kreuwel et al., 2001), were acquired from Jackson laboratory (Bar Harbor, Maine). All mice were maintained in AAALAC-approved barrier facilities and all experiments were conducted with the approval of the Institutional Animal Care and Use Committee (IACUC) at Drexel University. Influenza A- Puerto Rico/8/34 (PR8; H1N1) viruses were propagated in specific-pathogen-free eggs and stored at –80 °C for subsequent use. Mice were infected intravenously (i.v.) with 200 µl of sterile saline containing 300 Hemagglutination units (HAU) of PR8.

2.2. Cell preparation and adoptive transfer

Mice were sacrificed by CO₂ asphyxiation followed by cervical dislocation, and spleens were aseptically removed. Lymphocytes were isolated using 0.83% NH₄Cl from young and aged mice. Purification of CD8 T cells from TCR Tg mice (Clone-4 mice) and DCs from young wt BALB/c mice was performed by MACS using CD8a and CD11c Microbeads, respectively (Miltenyi Biotec, Bergisch Gladbach, Germany). The phenotype of the cells was determined by flow cytometry before and after purification. 1×10^6 enriched DCs (purity > 70%) were adoptively transferred or co-transferred i.v. with different doses of purified Tg CD8 T cells (purity > 92%) into BALB/c mice, respectively. Six hours after transfer, the recipients were infected with PR8 as described above.

2.3. Flow cytometry and FACS Analysis

Single-cell suspensions from the spleens were prepared and 1×10^6 splenocytes were stained for surface markers using mAbs. Anti-CD8, CD44, Thy1.1, and MHC I-A^d mAbs were purchased from BD PharMingen (San Diego, CA). H-2K^d HA₅₁₈₋₅₂₆ (IYSTVASSL) tetramer was obtained from NIAID MHC Tetramer Core Facility (Atlanta, GA). Intracellular IFN- γ staining was performed using anti-IFN- γ mAb (BD PharMingen) with the Cytotfix/Cytoperm kit (BD Pharmingen) after 5 h *in vitro* stimulation with or without HA₅₁₈₋₅₂₆ peptide (SynPep, Dublin, CA). The peptide was used at a concentration of 0.1 μ g/ml. Samples were acquired with a FACS CanTo (Becton Dickinson, San Jose, CA), and data were analyzed using FlowJo software (Tree Star, Inc., San Carlos, CA).

2.4. Statistical analysis

All statistical analyses were performed using Student's *t* test. Significant differences were determined at the level of $p < 0.05$. Results are expressed as mean $\pm$ SD.

3. Results

3.1. Effect of adoptively transferred DCs on expansion of young Tg CD8 T cells in aged mice

In a previous study, we found that the aged environment significantly inhibits clonal expansion of and IFN- γ production by specific Tg CD8 T cells of young mice during virus infection (Po et al., 2002; Jiang et al., 2009). To determine if the inability of the aged environment to support expansion of young Tg CD8 T cells was due to a deficiency of DCs, we co-transferred 1×10^4 purified TCR Tg CD8 T cells of young Clone-4 mice (Thy1.1⁺), which specifically recognize influenza virus HA₅₁₈₋₅₂₆ epitope, with enriched DCs of young BALB/c mice into congenic young and aged mice (Thy1.2⁺). The recipients were infected with influenza virus and the expansion of Tg CD8 T cells in the spleen was measured on Day 4 post infection. The function of the specific CD8 T cells was examined by intracellular IFN- γ staining after stimulation *in vitro* with HA₅₁₈₋₅₂₆ peptide. As shown in Fig. 1A, transfer of TCR Tg CD8 T cells alone resulted in limited expansion in aged compared to young mice (Aged vs young: percentage: $2.8\% \pm 0.5$ vs $5.5\% \pm 1.1$, $p < 0.05$; absolute number: $1 \times 10^5 \pm 0.4$ vs $3.9 \times 10^5 \pm 0.9$, $p < 0.05$). However, when DCs from young mice were co-transferred with Tg CD8 T cells, the expansion of the Tg CD8 T cells significantly increased in aged mice after infection (Percentage: $2.8\% \pm 0.5$ to $7.3\% \pm 0.4$, $p < 0.05$; absolute number: $1 \times 10^5 \pm 0.4$ to $2.2 \times 10^5 \pm 0.1$, $p < 0.05$), while neither percentage nor number of Tg CD8 T cells was increased in young recipients upon DC transfer (Percentage: $5.5\% \pm 1.1$ to $5.4\% \pm 0.3$, $p > 0.05$; absolute number: $3.9 \times 10^5 \pm 0.9$ to $4.0 \times 10^5 \pm 0.7$, $p > 0.05$). Similar to the expansion, the function of the Tg CD8 T cells was also enhanced by co-transfer of DCs into aged mice. After *in vitro* culture with HA₅₁₈₋₅₂₆ peptide, IFN- γ secreting Tg CD8 T cells significantly increased in both percentage of CD8 T cells ($2.7\% \pm 0.5$ to $7.6\% \pm 0.7$, $p < 0.05$) and absolute number ($0.75 \times 10^5 \pm 0.1$ to $1.9 \times 10^5 \pm 0.4$, $p < 0.05$) in aged mice with co-transfer of DCs (Fig. 1B). However, no significant

increase was observed in either percentage or number in young recipients. These results show that DCs from young mice can significantly enhance the specific Tg CD8 T cell response to virus infection in aged, but not young, mice, and suggest that the limited expansion in aged mice may be due to the decreased number of DCs and/or their impaired function *in vivo* with aging.

To examine whether co-transfer of DCs can support the expansion of a higher number of TCR Tg CD8 T cells, we transferred 100-fold more (1×10^6) Tg CD8 T cells with the same numbers of DCs used before (Fig. 1). The expansion and function of the Tg CD8 T cells in the spleen were examined on Day 3 after influenza infection. The higher numbers of Tg CD8 T cells transferred did not affect the results observed. In aged mice, co-transfer of DCs resulted in a three-to-four fold increase in the expansion of the Tg CD8 T cells (Fig. 2A) and their production of IFN- γ (Fig. 2B), while in young recipients the co-transfer of DCs had no impact on the expansion or function of Tg CD8 T cells. These data demonstrate that the DCs transferred into the aged mice have the capacity to support the response of large numbers of Tg CD8 T cells to virus infection, and further indicate that DCs of aged mice are defective in either quantity or quality.

3.2. Effect of adoptive transfer of DCs of young mice to aged mice on endogenous CD8 T cell response to primary influenza infection

Our previous studies demonstrated that specific CD8 T cell response to virus infection in aged mice is decreased compared to young mice (Po et al., 2002; Jiang et al., 2009). To determine whether the decreased CD8 T cell response could be increased with additional DCs, we adoptively transferred the same number of enriched CD11c⁺ splenocytes of young mice utilized in Figs. 1 & 2 into aged BALB/c mice. Influenza-specific CD8 T cells after virus infection were visualized by HA₅₁₈₋₅₂₆ tetramer staining. As shown in Fig. 3A and 3B, on Day 10 after influenza virus infection, the HA₅₁₈₋₅₂₆-specific CD8 T cell response was increased, though not significantly, when CD11c⁺ cells were adoptively transferred (–DCs vs +DCs: percentage: $0.32\% \pm 0.05$ vs $0.46\% \pm 0.09$, $p > 0.05$; absolute number: $1.2 \times 10^4 \pm 0.2$ vs $1.6 \times 10^4 \pm 0.1$, $p > 0.05$). These results suggest that the decreased virus-specific response of aged mice could not be overcome by the numbers of DCs transferred from young mice.

4. Discussion

It is known that CD8 T cell immune response to virus infection is impaired with aging (Po et al., 2002; Kapasi et al., 2002; Jiang et al., 2009; Brien et al., 2009), and both intrinsic and extrinsic factors contribute to this defect (Linton et al., 2005). Many studies have been performed *in vitro* to assess the mechanisms of age-related immunological alteration (Jiang et al., 2009; Jiang et al., 2003; Ahmed et al., 2009). Only limited *in vivo* evidence has been available to demonstrate that the decreased CD8 T cell response may result from either intrinsic or extrinsic deficiencies in CD8 T cell immunity (Linton et al., 2005; Jiang et al., 2009; Tesar et al., 2006; Yager et al., 2008). The enhanced expansion of TCR Tg CD8 T cells of young mice in aged recipients by co-transfer of DCs from young mice suggest that quantitative or qualitative changes in DCs of aged mice contribute to the limited response of aged mice. However, our results also demonstrate that the expansion of endogenous CD8 T cells is not increased significantly when DCs from young mice were transferred (Fig. 3). These data suggest that 1) the number of DCs transferred was insufficient to support the specific CD8 T cell response and/or 2) the intrinsic defect of CD8 T cells that occurs in aged mice cannot be overcome by DCs. Since the data of our adoptive co-transfer experiments (Figs. 1 & 2) suggest that the number of DCs transferred could significantly enhance the response of a large number of young Tg CD8 T cells in aged mice, it appears that the intrinsic defect of CD8 T cells may be the limiting component in the decreased immune response with aging (Figs. 1 & 2).

However, the design of the two studies may suggest a third possibility. Expansion of Tg CD8 T cells occurred when DCs were co-transferred with the CD8 T cells, allowing maximally interaction of the DCs and CD8 T cells. In contrast, in the endogenous response, it is necessary for the adoptively transferred DCs to migrate to the spleen and interact with the endogenous CD8 T cells. Since Linton et al have demonstrated altered migration of DCs in aged mice (Linton et al., 2005), it is possible that the *in situ* interactions were not optimal. Regardless of the mechanism, the current data illustrate an important point: if the intrinsic defects in aged T cells were corrected, the aged environment would still pose a block.

In summary, our current study shows that DCs of aged mice are an important limiting factor in the CD8 T cell immune response to specific antigen, in this case influenza virus. While the DCs of aged mice do not seem to be functionally deficient *in vitro*, their decreased number may play an important role in the impaired immune response of aged mice. Alternatively, their function or migration may be altered *in vivo*. In addition, our results are also consistent with the hypothesis that the decreased response of CD8 T cells of aged mice may result from an intrinsic defect that cannot be corrected by maximal DC interaction. These results emphasize the importance of characterizing the entire range of defects that limits the immune response before appropriate strategies can be developed to improve the response of the elderly.

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Abbreviations used

DC	dendritic cell
HAU	hemagglutination unit
Tg	transgenic
HA	hemagglutinin

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FIG. 1.

Enhancement of limited expansion of TCR Tg CD8 T cells in aged mice by DCs. 1×10^4 purified CD8 T cells from spleens of Clone-4 mice (Thy1.1⁺) were adoptively transferred or co-transferred with CD11c⁺ cells of young BALB/c mice (1×10^6 /mouse) into congenic young and aged mice (Thy1.2⁺). Recipient mice were infected i.v. with PR8 6 h after transfer. On Day 4 post-infection, splenocytes were isolated and stained with antibodies to CD8, CD44, and Thy1.1. Tg CD8 T cells were identified by Thy1.1⁺CD44^{high} staining after gating on total CD8 T cells. The function of the specific CD8 T cells was examined by intracellular IFN- γ staining after *in vitro* stimulation with HA₅₁₈₋₅₂₆ peptide for 5 h. (A) Percentages of donor Tg CD8 T cells (Thy1.1⁺) of total CD8⁺ cells, and absolute numbers of CD8⁺Thy1.1⁺ cells. (B) Percentages of Thy1.1⁺IFN- γ ⁺ cells gated on CD8⁺ cells, and absolute numbers of Thy1.1⁺IFN- γ ⁺ cells. Each plot is representative of one mouse with numbers reflecting $\pm$ SD of 3–4 mice. * $p < 0.05$. Results are representative of three independent experiments with similar results.

FIG. 2.

DCs enhance the response of a large number of Tg CD8 T cells in aged mice. Similar to Fig. 1, except: 1) 1×10^6 purified CD8 T cells from spleens of Clone-4 mice were adoptively transferred; and 2) splenocytes were isolated on Day 3 post-infection. (A) Percentages of Tg CD8 T cells of total CD8⁺ cells, and absolute numbers of CD8⁺Thy1.1⁺ cells. (B) Percentages of Thy1.1⁺IFN- γ ⁺ cells gated on total CD8⁺ cells, and absolute numbers of Thy1.1⁺IFN- γ ⁺ cells after *in vitro* stimulation with HA₅₁₈₋₅₂₆ peptide. Each plot is representative of one mouse with numbers reflecting $\pm$ SD of 3–4 mice. * $p < 0.05$. The experiment was performed three times with similar results.

FIG. 3.

DCs provide limited enhancement of endogenous CD8 T cell response in aged mice. Enriched CD11c⁺ cells of spleens of young BALB/c mice (1×10^6 /mouse) were adoptively transferred into aged congenic mice. Mice were infected i.v. with PR8 6 h later. On Day 10 post infection, splenocytes were isolated, stained with CD8, CD44, and K^d-HA₅₁₈₋₅₂₆ tetramer, and assessed by flow cytometry. Tetramer⁺/CD44^{high} cells are presented as percent (A), and absolute number (B) of total CD8 T cells. Each plot shows CD8 T cells from one representative mouse with numbers indicating mean $\pm$ SD of 3–4 mice. * $p < 0.05$. Data are representative of four independent experiments with similar results.