Age-Related Loss of CD62L Impairs Lymph Node CD4 T Cell Mobilization

Bo-Chin Chiu[1], Valerie R. Stolberg[2] and Stephen W. Chensue*[,2]

Department of Pathology[1], University of Michigan Medical School, Ann Arbor; and the Department of Pathology and Laboratory Medicine[2], VA Ann Arbor Healthcare System, Ann Arbor, Michigan, USA

Abstract: Impaired pulmonary immunity in the aged is poorly understood. Lymph node recirculation of CD4+ T cells is important for adaptive immunity and depends on CD62L and CCR7. We compared circulatory capacity of CD4+ T cells from young and aged mice *in vivo*. When congenic CD4+ T cells from young donors were transferred to recipients, the frequencies of donor among endogenous CD4+ T cells was equivalent in sampled tissues. In contrast, those from old donors were five times lower in mediastinal lymph nodes (MLN) than in the spleen. When mixed and transferred to recipients, young CD4+ T cells were four times more abundant in MLN of recipients than old CD4+ T cells. Following pulmonary challenge, old CD4+ T cells were four times less frequent in the MLN than in the spleen. Compared to young, old CD62L^{+}CD4+ T cells maintained high levels of CCR7 expression but suffered a 50% loss of CD62L. Thus, loss of CD62L, but not CCR7 expression by aged CD4+ T cells is a major factor for impaired lymph node mobilization.

Keywords: Aging, T helper cell, lung, CD62L, chemokine receptors.

INTRODUCTION

Aging is associated with increased susceptibility to infectious diseases, among which respiratory infections are particularly prominent. Influenza and pneumococcal pneumonia are infections that have a higher fatality rate in older than in younger people [1]. Latent tuberculosis, which is a pulmonary mycobacterial infection under the control of persistent T cell-mediated immunity, can reactivate in the aged [2]. During the recent outbreak of severe acute respiratory syndrome, the age dependence of disease severity and mortality was impressive. In Hong Kong, mortality rates of affected individuals of 0−24, 25−44, 45−64 and >65-years old were respectively 0%, 6%, 15% and 52%,(www.who.int/csr/sars/en/WHOconsensus.pdf). This progressive age dependence in mortality is not totally explained by co-morbid factors, and the underlying mechanisms remain unclear. Our understanding of the effect of aging on respiratory immunity is still rudimentary. When assessed *in vitro*, age-associated changes have been reported in nearly all cellular components of the immune system, and the general consensus is that immune function declines with age (reviewed by Miller [3] and Effros [4]). However, the relationship between these changes and the protective immunity has not been fully addressed. Events and parameters that define protective respiratory immunity have not been evaluated in a systematic way under *in vivo* conditions.

Local protective immunity depends on immune surveillance by lymphocytes. Although all types of leucocytes can leave the blood and enter tissues, only lymphocytes recirculate through lymph nodes and return to the blood [5]. Rapid recirculation enables small numbers of Ag-specific lymphocytes to be quickly brought into contact with antigen presenting cells. Both naïve and central memory but not effector T cells recirculate through lymph nodes in search of antigen presenting cells [6]. Although effector T cells provide rapid protective immunity without recirculation, they have limited proliferative capacity [6] and are unable to confer long-term memory [7]. Thus, T cell recirculation is important for establishing both primary and memory T cell responses.

Each day the lung is exposed to more than 10,000 liters of air, which may contain hazardous chemicals, microorganisms, as well as environmental antigens. Therefore, respiratory exposure represents a major challenge for the aging immune system. Mechanisms have evolved to eliminate most pathogens locally in the lung, preventing them from spreading systemically. In the mouse, it is generally accepted that adaptive immunity to respiratory antigen challenge is initiated in draining mediastinal lymph nodes (MLN) [8]. Under homeostatic conditions, the MLN is normally in a resting condition with little proliferative activity. The node is normally populated by antigen presenting cells as well as recirculating resting state T and B cells [9]. An activated draining lymph node is characterized by a dramatic increase in cellularity. Effector CD4 T cells are generated from precursor cells that include naïve and central memory CD4 T cells during primary and secondary immune responses in MLN. Efficient antigen delivery and Th cell recirculation to lymph nodes is essential for protective pulmonary immunity. It has been shown that antigen-loaded T-zone dendritic cells appear in the draining lymph node within hours of antigen deposition in the lung [10]. In addition, CD4 T cells have been shown to enter peripheral lymph nodes almost immediately after adoptive transfer [11].

The role of L-selectin and CCR7 in CD4 T lymphocyte recirculation is well established [12]. Aging is reportedly associated with a loss of constitutive expression of L-selectin and CCR7 by CD4 T cells [13, 14]. However, the effect of aging on Th cell recirculation has not been reported. In the present study, we conducted adoptive transfer experiments in mice to study the effect of aging on CD4 T cell recirculation

*Address correspondence to this author at the University of Medical School, Ann Arbor; and the Department of Pathology and Laboratory Medicine, VA Ann Arbor Healthcare System, Ann Arbor, Michigan, USA; E-mail: schensue@med.umich.edu

in pulmonary draining lymph nodes and other lymphoid tissues under both inflammatory and non-inflammatory conditions. In addition, we examined the relative contribution of L-selectin and CCR7 to migration patterns.

MATERIALS AND METHODS

Mice

Male C57BL/6 mice of indicated ages were obtained from the National Institute of Aging contract colony at Harlan Laboratories (Indianapolis, IN). Male C57BL/6 congenic for the CD45.1 (B6.SJL-*Ptprc^{a}Pep3^b/BoyJ*) and CD90.1 (B6.PL-*Thy1^a/CyJ*) markers were purchased from the Jackson Laboratory (Bar Harbor, ME) and used at 3 to 4 months of age. Mice were maintained under specific pathogen-free conditions and provided food and water ad libitum. Necropsy examinations were performed at the time of mouse sacrifice in order to exclude individuals with organ failure or neoplasms. The University of Michigan Committee on Use and Care of Animals approved all animal studies.

Cell Preparation and Flow Cytometry

Single cell suspension were prepare from lung as described previously[15]. Briefly, after perfusion with cold RPMI 1640, lungs were excised, placed in cold RPMI 1640 medium, and then homogenized in a Waring blender. Homogenates were incubated with digestion medium containing RPMI (JRH Biosciences, Lenexa, KS), 10% FBS (Intergen, Purchase, NY), 10 mM glutamine and 1000 U/ml type IV collagenase (Sigma Chemical Co., St. Louis, MO). The digest was sieved through a stainless steel mesh (#100) and then washed four times by centrifugation in RPMI1640. Single cell suspensions were also prepared from freshly harvested lymph nodes as previously described [15]. Monoclonal antibodies used for mouse T cell analysis included FITC-, PE- and PE-Cy5-conjugated anti-CD4 (GK1.5), anti-CD69 (H1.2F3), anti-CD44 (IM-7), anti-CD25 (PC61), anti-CD8a (53-6.7), anti-CD62L (MEL-14), anti-CD90.1 (HIS51) and anti-CD90.2 (53-2.1) (all from BD PharMingen San Diego, CA). Anti-CCR7 was purchased from eBioscience. All isotype controls and anti-CD16/CD32 (2.4G2) were also from BD Pharmingen. After blocking with anti-CD16/CD32 for 5 minutes, cells were stained with fluorescent-labeled antibodies or isotype control antibodies in 2% FBS-PBS buffer. A FACScan flow cytometer with CellQuest software (B-D, San Jose, CA) was used for data acquisition and analysis.

Cell Isolation, Labeling and Transfer

CD4 T cells were purified from spleens of individual donor mice by labeling cells with MACS anti-CD4 microbeads (Miltenyi Biotec, Auburn, CA) and positively selected. In all experiments more than 95% of recovered cells were CD4 positive. For CD62L+ CD4 T cell enrichment, splenocytes were first enriched for CD4 T cells with MACS CD4 T cell enrichment kit (negative selection kit). The CD4 T cells were then labeled with PE-anti-CD62L and sorted into CD62L$^+$ and CD62L$^-$ populations with MACS anti-PE micro-beads. The enrichment of the recovered cells was confirmed by flow cytometric analysis and was routinely greater than 90%. For CFSE labeling, cells were resuspended at a concentration of 10^7/ml in PBS containing 0.01% BSA. CFSE (Molecular Probes Inc., Eugene, OR) was added to single cell suspension at a final concentration of 5 μM and incubated for 10 minutes at 37°C. After incubation, the cells were washed once with complete RPMI, incubated for 5 minutes at 37°C with complete RPMI, and washed once with PBS. The cells were then checked for viability and fluorescent labeling using a fluorescent microscope. Five to 10 million viable cells were transferred intravenously into each recipient mouse.

Real-Time RT-PCR Analysis

Real-time RT-PCR analyses were performed as described [16]. Poly(A) pure mRNA was isolated from cells using mRNA isolation kits (Ambion, Austin, TX) and reverse-transcribed using Reverse Transcription System kits (Promega, Madison, WI). Real-time PCR was performed on samples before and after reverse transcription to assess genomic DNA contamination levels in a sample preparation. DNase treatment was applied whenever contamination was detected. The ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA) was used for real-time PCR analysis with comparative CT method. TaqMan (Applied Biosystems) predeveloped reaction kits were used. In all cases, the thermal cycling condition was programmed according to the manufacturer's instructions. Data are expressed as arbitrary units as described previously [15].

Statistics

Analysis of variance (ANOVA) was used for intergroup comparisons with P<0.05 considered to indicate significance. All observations were repeated at least twice in separate experiments.

RESULTS

Adoptive Transfer Model for CD4 T Cell Recirculation Analysis

In young mice, T cells recirculate throughout the body as part of immune surveillance. It has been demonstrated in parabiotic mouse models that the ratios of host to donor T cells in the spleen, PLN and lung of either partner reach 1:1 as soon as the ratio in the blood circulation reaches 1:1 [17]. These data suggest that there are very few long-term resident T cells in these tissues, and that instead the T cell populations in these tissues are maintained by a rapidly recirculating pool. In order to establish the validity of our adoptive transfer approach, we attempted to repeat this observation.

When CD4 T cells from spleens of young C57BL6, identified either by congenic CD90.1 marker or by CFSE labeling, are injected intravenously into young or old hosts, the donor cells can be identified in recipients and their frequencies determined by flow cytometric analysis. Using this approach, we conducted quantitative studies using young donor CD4+ T cells and young recipients. Frequencies of donor CD4+ T cells among total gated CD4+ T cells in spleen, axillary lymph node (ALN), lung and the draining mediastinal lymph node (MLN) two days after cell injection were determined using flow cytometry (Fig. **1**). As expected, the frequencies were comparable in all sampled tissues. Similar results were found when donor cells were derived from peripheral lymph nodes (data not shown). These data showed that adoptively transferred CD4+ T cells can be effectively tracked by flow cytometric analysis and they recirculate

without barrier in young mice as previously reported using parabiotic mouse models [17].

Aged CD4 T Cells Display Impaired Recirculation Through Peripheral Lymph Nodes Under Steady State Conditions

Because CD4+ T cells recirculated freely through tissues, we surmised that the frequency of old donor CD4+ T cells among the endogenous CD4+ T cells in a particular organ might be used as a measure of migration efficiency of the donor cells to a particular organ. With this assumption and using adoptive transfers, we analyzed migration of CD4 T cells from 24-month-old C57BL6/NIA mice. Two days after adoptive transfer, the frequencies of the donor CD4 T cell among total CD4 T cells in the spleen, ALN, lung and MLN were measured. Upon analysis, age-associated changes in CD4+ T cell recirculation pattern became clear. The frequency of transferred cells was 5 fold higher in the spleen and lung than in the ALN and MLN (Fig. **2a**). Because all endogenous CD4 T cells are in rapid recirculation in the young recipients, the data indicate that aged CD4 T cells are 5 times less likely to recirculate through peripheral lymph node (PLN) than spleen. However, there was no loss of ability of aged CD4 cells to circulate through the lung.
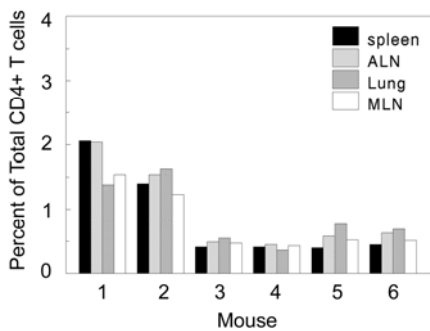

Fig. (1). Young CD4+ T cells recirculate through the lung and mediastinal lymph node without barrier. Splenocytes from young C57BL6 mice were labeled with CFSE and were injected I.V. into young C57BL6 mice; 40 h after transfer, spleen, axillary lymph node, lung and mediastinal lymph node were analyzed for numbers of CFSE+CD4+ T cells by flow cytometry. The number of donor CD4 T cells is presented as the percent of total CD4 T cells recovered from the respective tissues of individual mice (horizontal axis).

To more accurately quantify the number of aged CD4 T cells that are able to recirculate through peripheral lymph nodes we injected young and old CD4 T cells at 1:1 ratio into young recipients. Donor cells were identified by CFSE label, and young donor cells were differentiated from old donor cells by their expression of the congenic marker CD90.1. The ratios of old to young donor CD4+ T cells were determined in the spleen and MLN of the recipients two days after injection by flow cytometry. In spleens, the mean of the ratios from 6 individual recipients was 1.2 to 1. In MLN, the mean was 0.24 to 1 (Fig. **2b**).

These data demonstrated that CD4+ T cells from old mice did not recirculate efficiently through MLN. It should be noted that old and young donor spleens contained nearly equal numbers of CD4+ T cells in all of the experiments.
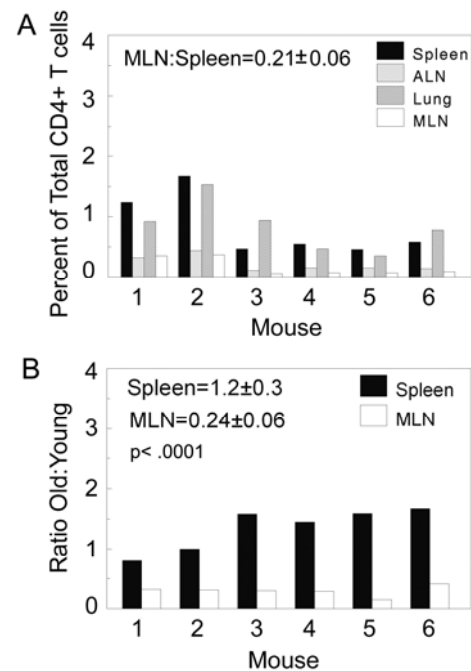

Fig. (2). CD4+ T cells migration through mediastinal lymph node is diminished by aging. (**A**) Splenocytes from 24-month-old C57BL/6 mice were labeled with CFSE and were injected I.V. into young C57BL/6 mice; 40 h after transfer, spleen, ALN, lung and MLN were analyzed for the number of CFSE+CD4+ T cells by flow cytometry. The number of donor CD4+ T cells is presented as the percent of total CD4+ T cells recovered from the respective tissues of individual mice (horizontal axis). Numbers above indicate the mean and standard deviation of the ratio of the frequency of the donor CD4+ T cells in MLN to that in spleen. (**B**) CD4 T cells from 24-month-old C57BL/6 CD90.2 mice were mixed with CD4 T cells from 4-month-old C57BL/6 CD90.1 congenic mice at 1:1 ratio, labeled with CFSE and injected I.V. into young C57BL/6 mice; 40 h after transfer, spleen and MLN were analyzed for the number of CFSE+CD4+ CD90.2+ and CFSE+ CD4+CD90.1+ T cells by flow cytometry. The ratio of the number of old to young CD4+ T cells recovered from the spleen is compared with that from MLN of individual mice (horizontal axis). Numbers above indicate the mean and standard deviation of the ratios in MLN and spleen.

Aged CD4 T Cells Display Impaired Lymph Node Mobilization Under Inflammatory Conditions

Despite dramatic age-associated changes in lymph node migration, our data showed that, under steady state conditions, CD4+ T cell trafficking through the lung was not affected by aging (Fig. **2a**). Unlike secondary lymphoid tissues, tertiary tissues such as bronchial associated lymphoid tissue are low traffic sites for T cells. CD4+ Th cells are far less numerous in the lung than in lymphoid tissues. However, the number of T cells increases dramatically in lungs and draining lymph nodes during inflammation [18]. Whereas recruitment of T cells by high endothelial venules (HEV) in PLN is constitutive, recruitment of T cells in the lung depends on inflammatory mediators. We next studied the effect of aging on CD4+ T cell trafficking in the lung under inflammatory conditions. Young recipient mice were challenged with intranasal administration of LPS in PBS on 2 consecutive days before receiving donor cells. The degree
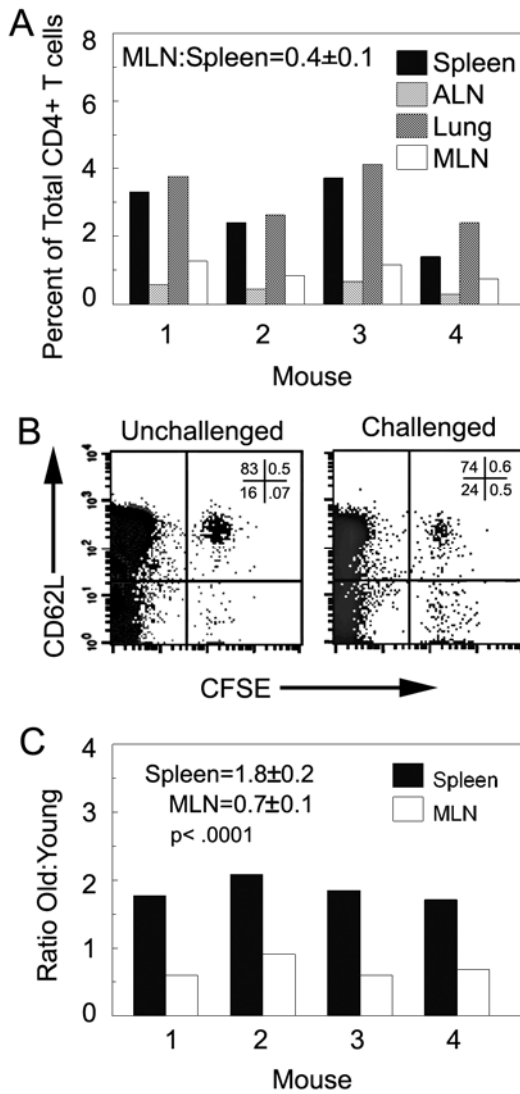
Fig. (3). CD4+ T cells migration through mediastinal lymph node under inflammatory conditions is diminished by aging. Young C57BL/6 mice were challenged intranasally with LPS on two consecutive days. Splenocytes from aged C57BL/6 mice were labeled with CFSE and were injected I.V. into challenged mice on day-2; 40 h after transfer, spleen, ALN, lung and MLN were recovered. (**A**) The number of CFSE+CD4+ donor T cells, determined by flow cytometry, is presented as the percent of total CD4+ T cells recovered from the respective tissues of individual mice (horizontal axis). Numbers above indicate the mean and standard deviation of the ratio of the frequencies in MLN and spleen. (**B**) Gated CD4 T cells from MLN were analyzed for CD62L expression. Note, CD62L- donor CD4 T cells (CFSE+) were excluded from MLN in unchallenged mice (lower right quadrants), but were found in MLN of challenged mice. (**C**) CD4 T cells from 24-month-old C57BL/6 CD90.2 mice were mixed with CD4 T cells from 4-month-old C57BL/6 CD90.1 congenic mice at 1:1 ratio, labeled with CFSE and injected I.V. into the young intranasal LPS challenged C57BL/6 mice on day-2; 40 h after transfer, spleen and MLN were analyzed for the number of CFSE+CD4+ CD90.2+ and CFSE+CD4+ CD90.1+ T cells by flow cytometry. The ratio of the number of old to young CD4+ T cells recovered from the spleen is compared to that from MLN of individual mice (horizontal axis). Numbers above indicate the mean and standard deviation of the ratio in MLN and spleen.

of inflammation was determined by flow cytometry of lung-digested cells at the end of the experiment. The number of neutrophils, identified as CD11b+Gr-1hi, increases rapidly following the LPS challenge in the lung, confirming the LPS induced inflammation. Despite the increased traffic of leukocytes in the inflammatory lungs, the frequencies of donor old CD4+ T cells in the lung and in the spleen were similar (Fig. **3a**). These data suggest that young and old CD4+ T cells have similar capacity to circulate through the lung under inflammatory and non-inflammatory conditions. Notably, the frequencies of old donor CD4+ T cells in MLN, which drained the inflammatory site, were consistently about 2-fold higher than in the non-draining ALN (Fig. **3a**). Furthermore, old donor CD4+ T cells recovered from the draining MLN included some CD62L- donor CD4+ T cells, which are usually excluded from PLN under non-inflammatory conditions (Fig. **3b**). However, despite the activation of this alternative pathway of CD4+ T cell trafficking through afferent lymph, CD4+ T cells from old mice were still 3 times less likely to enter the MLN than the spleen or lung (Fig. **3a**). Finally, when CD4 T cells from young and old donors were mixed at 1:1 ratio before injection, young CD4 T cells were much more likely to be found in the MLN, whereas the old CD4 T cells were more likely to be found in the spleen (Fig. **3c**). Thus, aging also compromises CD4+ T migration to MLN under inflammatory conditions.

CD62L+ Cells From Old Mice Display Impaired Lymph Node Localization

It has been previously shown that in young mice most CD4 T cells from the spleen express high levels of CD62L, whereas in aged mice a large proportion of these cells lose CD62L expression [13], and CD4+ T cells from CD62L deficient mice are unable to enter peripheral lymph node *via* HEV [19]. We wished to evaluate the role of CD62L and aging in CD4+ T cell recirculation. First, we found that, even though only one third of the injected donor CD4+ T cells from aged mice were CD62L+ (Fig. **4a**), those recovered from the lymph nodes of the recipients were about 85% CD62L+ (Fig. **4b**). In the spleen, however, the proportion recovered CD62L positive and negative donor cells was largely unchanged compared to the injected cells (Fig. **4b**).

We next separated CD62L+ from the CD62L− cells using a magnetic bead system. Flow cytometry data confirmed that the separation was successful (Fig. **5a**). When CD62L− CD4+ T cells from either young or old donor mice were injected into young recipient mice, very few were found in MLN, but they were found in the spleen (Fig. **5b**, right panel). When young CD62L+CD4+ T cells were transferred to young recipients, the frequencies of donor cells were nearly equal in the two tissues. In contrast, when old CD62L+ CD4+ T cells were transferred, the donor CD4+ T cells were still 3 times more likely to be found in spleen than in MLN (Fig. **5b**, left panel), indicating that even CD62L+ CD4+ T cells in old mice were defective in recirculation. It should be noted that heterozygous CD62L knockout lymphocytes, which express lower levels of surface CD62L than wild type cells exhibit impaired recirculation function [20]. Therefore, it is possible that only cells expressing the highest levels of CD62L are able to recirculate efficiently through HEV. In this regard, although average intensity of CD62L+ cells from old mice was comparable to that of young mice,

there was downward skewing of the expression curve (Fig. **5a**).
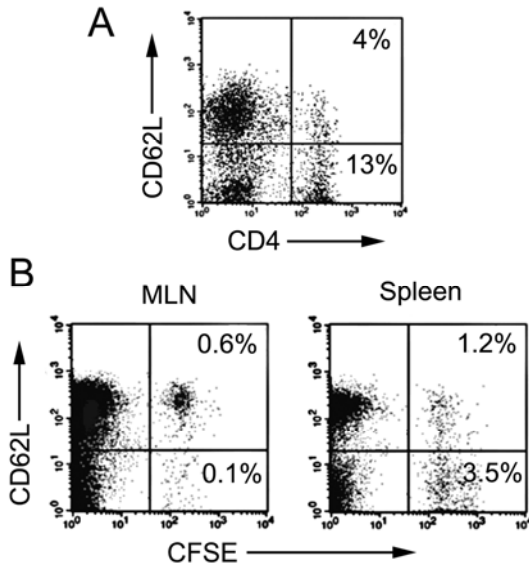

Fig. (4). CD62L expression is necessary for aged CD4+ T cells to recirculate through peripheral lymph nodes. Splenocytes from 24-month-old C57BL6 mice were labeled with CFSE and injected I.V. into young C57BL6 mice; 40 h after transfer, cells from spleen and mediastinal lymph node were analyzed for CD62L expression. (**A**) Flow cytometry of the total injected cell population. (**B**) Flow cytometry of gated CD4+ T cells of the recovered cells from indicated tissues of the recipient. Numbers in quadrants indicate the percentage of cells in the respective areas.

Loss of CD62L but not CCR7 Expression by Aged CD4 T Cells is Responsible for Impaired Lymph Node Mobilization

Both naïve and central memory CD4+ T cells can recirculate through lymph nodes [6]. CD4+ T cell recirculation reportedly requires functional expression of both CD62L and CCR7 [12].

Importantly, the level of CD62L expression determines the efficiency of the recirculation such that a 50% reduction in CD62L expression by lymphocytes from heterozygous CD62L knockout mice results in a 50 to 70% decrease in short term lymphocyte migration into PLN relative to wild-type lymphocytes [19]. Moreover, the number of CD62L+ CD4+ T cells decreases progressively with age in mice [13]. However, variance of CD62L expression by the CD62L$^+$ CD4+ T cells has not been fully appreciated. In search of potential mechanisms for the age-related recirculation impairment of CD62L$^+$ cells, we compared CD62L$^+$ CD4 T cells from different age groups for CD62L and CCR7 expression. The difference in CCR7 protein and transcript expression levels was very dramatic between CD62L+ and CD62L- fractions of CD4 T cells (Figs. **6a** and **6c**). Specifically, young CD4+ T cells populations were dominated by CCR7+CD62L^{hi} cells, presumably naïve T cells. As mice aged, there appeared a second population of CCR7-CD62L-cells, consistent with non-lymph node homing effectors T cells. Interestingly, CCR7 levels were comparable to young mice among aged CD62L$^+$CD4+ T cells for both mRNA and surface protein expression (Figs. **6b** and **6c**). In contrast to

CCR7, the intensity of CD62L expression by specifically gated CCR7+CD62L$^+$ CD4+ T cells, possibly residual naïve T cells, decreased progressively with age (Fig. **6b**). We conclude that reduced CD62L expression among CCR7+ cells is largely responsible for impaired CD4+ T cell migration to MLN by CD4+ T cells in aged mice.
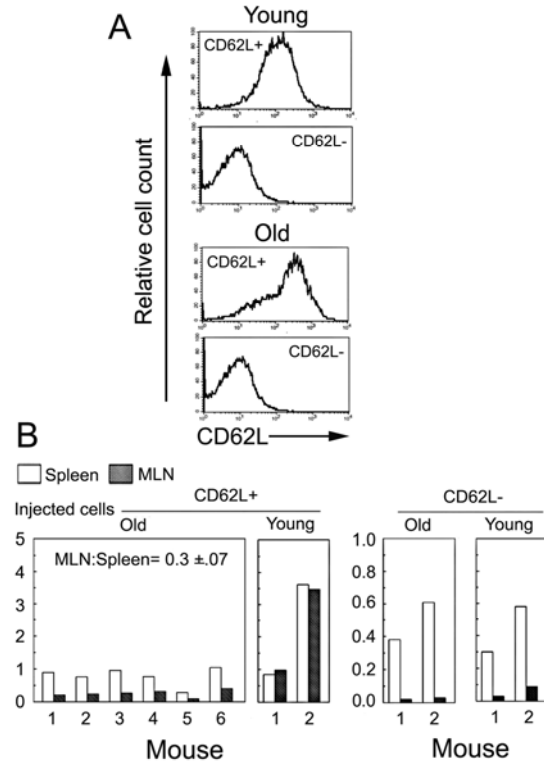

Fig. (5). CD62L expression is insufficient for aged CD4+ T cells to recirculate through peripheral lymph nodes. Splenocytes from young or old C57BL6 mice were sorted into CD62L$^+$ and CD62L$^-$ fractions using MACS beads, labeled with CFSE, and injected I.V. into young mice; 40 h after transfer, the number of donor CFSE+CD4+ T cells in spleen and MLN were compared. (**A**) Flow cytometry of the injected cells. Upper and lower panels show the injected CD62L$^+$ and CD62L$^-$ cell fractions. (**B**) Flow cytometric analysis of CD4+ T cells recovered from the spleen and MLN of the recipients. The number of donor CD4+ T cells is presented as the percent of total CD4+ T cells recovered from the respective tissues of individual mice (horizontal axis). Data are from three independent experiments with two mice each for old CD62L+ donor cells and from 2 independent experiments with 1 mouse each for other types of donor cells. Numbers above indicate the mean and standard deviation of the ratio of the frequency of the donor CD4+ T cells in MLN to that in spleen.

DISCUSSION

The results presented here reveal a potential mechanism for age-related immune compromise. Specifically, reduced CD62L expression by the CD62L+CCR7+CD4+ T cell subset can contribute to impaired CD4+ T cell recirculation and immune surveillance in aged mice. Surface CD62L expression is critical for efficient CD4+ T cell recirculation [19]. High levels of surface CD62L on CD62L+ CD4+ T cells in young mice confer the ability to recirculate efficiently. Thus, entry into peripheral lymph nodes by young naïve T cells is

barrier-free. Gene knockout models show that a 50% decrease in CD62L results in a 50 to 70% decrease in T cell recirculation efficiency [19]. We show that that the level of surface CD62L on the CD62L+ CD4+ T cell subset decreases progressively with age, such that by 24 months there is a 50% decrease in surface CD62L level and a 70% decrease in recirculation efficiency. With regard to lung immunity, the CD62L^{lo} T cells were excluded from mediastinal lymph nodes under homeostatic and inflammatory conditions. Although some CD62L-CD4+ T cells entered mediastinal lymph nodes under inflammatory conditions, the efficiency was low. In young mice the CD62L-CD4+ T cells represent a minor population, whereas in old mice the proportion of CD4+ T cells excluded from mediastinal lymph node increases, correlating with decreased CD62L expression. Our data also rule out any compromise of CCR7 expression, which could not compensate for the loss of CD62L.
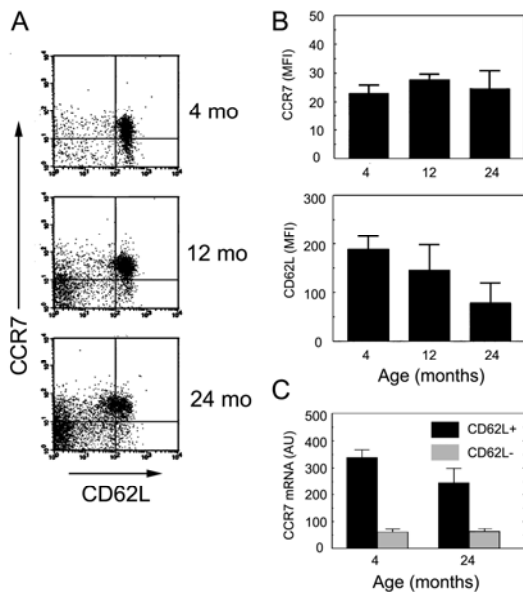

Fig. (6). Age-dependent loss of CCR7 and CD62L expression by CD4 T cells. (A) Surface expression of CCR7 and CD62L by CD4+ cells from spleens of mice of indicated age was determined by flow cytometry. Note the correlation between the expression of CCR7 and CD62L. (B) The mean fluorescent intensity (MFI) of CCR7 and CD62L of the CCR7 CD62L double positive CD4 T cells is determined and presented as mean and standard deviation. The effect of aging on CD62L MFI is clearly seen, P<0.001 (one-way ANOVA). Data are representative of two independent experiments with two mice each. (C) CD4 T cells from spleens of mice of indicated age were separated into CD62L+ and CD62 fractions. The cell fractions were analyzed for CCR7 mRNA expression by real time RT-PCR. Data are representative of two independent experiments with two mice each.

The terms, naïve and memory cells, have been used rather indiscriminately in the literature to describe T cells without consideration of whether the cells are actually antigen-experienced. CD62L negativity has been associated with memory phenotype, while naïve T cells are CCR7+ CD62L^{high}. Antigen-experienced memory CD4+ T cells are heterogeneous in their CD62L surface antigen expression and have different migratory capacity. Central memory T cells recirculate similarly to naïve T cells by virtue of their expression of CD62L and CCR7. Other cognate T cells persist as effector memory T cells, which lose constitutive expression of CCR7-CD62L- and are excluded from secondary lymphoid tissues. The persistence of effector memory T cells may depend on the presence of antigen, at least in the mouse [21, 22]. It has been shown that in the absence of antigen stimulation, antigen-experienced T cells maintain a central memory phenotype (CCR7+CD62L^{+}) indefinitely [23]. Thus, in the absence of acute and chronic immune responses, most CD4+ T cells should be CD62L^{+} and home to secondary lymphoid tissues. In young mice this is the status quo since most CD4+ T cells express high levels of CD62L. In fact, even in 4-month old mice, most CD4+ T cells are CD62L high. Thus, the loss of CD62L expression by CD4+ T cells seemed to accelerate late in life and our findings suggest that this is an effect on populations with a CCR7+CD62L+ phenotype.

Although CD62L-CCR7- CD4+ T cells observed in older mice enter the spleen efficiently, their lack of CCR7 expression may affect their homing to specific microenvironments in the spleen, as reported for CCR7 knockout T cells [24]. It will be of interest to determine to what extent these cells participate in immune responses, if they fail to migrate to the T cell zones of secondary lymphoid tissue. The loss of CD62L and recirculation capacity with age may be particularly characteristic of the CD4 subset of T cells. In our initial adoptive transfer experiments we also determined the frequencies of donor CD8+ T cells and B cells in different tissues of the recipients. Their frequencies did not seem to be affected by the age of the donor cells (data not shown).

T cell recirculation through peripheral organs is an integral part of immune surveillance. Although the constitutive throughput of T cells in the lung is low, traffic through sites of inflammation can be 30 times higher [18]. Our findings showed that traffic to the lung by aged CD4+ T cells was similar to young CD4+ T cells under homeostatic and inflammatory conditions. Previous studies have reported that young and aged lymphocytes migrate similarly to peripheral organs upon adoptive transfer under homeostatic conditions [25]. Our results would also be consistent with findings that neither CD62L nor G-protein coupled receptors are required for the migration of T cells to liver and lung [17].

Lymphocyte recirculation studies using *in vivo* models are rare in the aging immunology literature. One previous study showed that entry into spleen of aged mice by either young or old lymphocytes is inhibited [25]. One possible explanation is that in aged mice lymphocytes become stationary in the spleen and are difficult to displace. Although not the focus our study, we found in all experiments that aged donor T cells tended to be found in the spleen of the recipients. When young and old CD4+ T cells were co-transferred, the ratio of old to young CD4+ T cells was always higher in spleen than in blood (data not shown). However, it is not clear whether old T cells were actively circulating through or retained in the spleen. In any case, old CD4+ T cells have a competitive advantage in occupying the spleen, possibly because they are excluded from peripheral lymph nodes.

CONCLUSION

To date, there are very few studies that are able to make the link between aged-related changes and the susceptibilities to clinical illnesses that increase with age. Respiratory infections are among such illnesses. It has been demonstrated in young mice that there are few long-term resident T cells in mediastinal lymph nodes and lung [17]. Rather, a single rapidly recirculating T cell pool maintains the T cell populations in these tissues. T cell-mediated pulmonary immunity depends critically on T cell recirculation through the lung as well as draining nodes. The defects we have identified in our animal studies provide a mechanism to explain age-related respiratory immune compromise. Because CD4+ T cells from aged humans display similar phenotypic changes, it is tempting to speculate that the same mechanism is responsible in part for respiratory immune compromise in aged humans.

ACKNOWLEDGEMENTS

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