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T-Lymphocytes Expressing CC Chemokine Receptor-5 Are Increased in Frail Older Adults

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Abstract

OBJECTIVES—To evaluate the frequencies of T-lymphocytes expressing CC chemokine receptor-5 (CCR5⁺ T-cells) and their relationship with frailty in older adults.

DESIGN—Case-control study with an age-, race-, and sex-matched design.

SETTING—General Clinical Research Center.

PARTICIPANTS—Community-dwelling adults aged 72 and older from Baltimore, Maryland.

METHODS—Frailty was determined using five validated criteria: weakness, slow walking speed, fatigue, low physical activity, and weight loss. Those meeting three or more of these five criteria were defined as frail and those with none as nonfrail. Complete blood counts were performed to obtain peripheral lymphocyte counts using an automated (Coulter) counter. Peripheral blood was collected for surface immunofluorescent staining of CCR5 and other T-cell markers.

RESULTS—Twenty-six frail and matched nonfrail participants (mean age $\pm$ standard deviation 83.8 $\pm$ 5.3, range 72–94) completed the study. Frail participants had higher CCR5⁺, CCR5⁺CD8⁺, and CCR5⁺CD45RO⁺ T-cell counts than matched nonfrail controls (349 $\pm$ 160/mm³ vs 194 $\pm$ 168/mm³, P = .02; 208 $\pm$ 98/mm³ vs 105 $\pm$ 62/mm³, P = .02; and 189 $\pm$ 149/mm³ vs 52 $\pm$ 36/mm³, P = .01; respectively). Furthermore, there was a trend toward graded increase in these T-cell counts across the frailty scores in frail participants (e.g., CCR5⁺CD8⁺ counts of 123 $\pm$ 52/mm³, 248 $\pm$ 115/mm³, and 360 $\pm$ 215/mm³ for those with frailty scores of 3, 4, and 5, respectively).

CONCLUSION—These initial results suggest an expansion of the CCR5⁺ T-cell subpopulation in frailty. They provide a basis for further characterization of CCR5⁺ T-cells and their role in frailty, with potential therapeutic implications.

Keywords

frailty; inflammation; CCR5⁺ T-lymphocytes

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Frailty is an important geriatric syndrome characterized by poorer physiological reserve and greater vulnerability to severe adverse outcomes, including disability, dependency, and early mortality.^{1–3} Significant evidence suggests that chronic inflammation, marked by high interleukin-6 and C-reactive protein levels and increased white blood cell counts as a key pathophysiological factor in the syndrome of frailty.^{2,4–7} This chronic inflammation can contribute to frailty directly or through other intermediary processes, including hematopoietic and endocrine processes,^{4–7} but potential underlying immune mechanisms that contribute to frailty and its associated chronic inflammation in older adults have not been elucidated.

CC chemokine receptor 5 (CCR5) interacts with the chemokines CCL3, 4, 5, and 8 and plays an important role in regulating leukocyte recruitment, trafficking, and immune activation.^{8,9} It has been shown that CCR5⁺ T-lymphocytes have a type-1 proinflammatory phenotype^{10–12} and that CCR5⁺ T-lymphocytes can contribute significantly to several inflammatory conditions.^{9–13} In addition, CCR5 is a well-known co-receptor for human immunodeficiency virus type-1 (HIV-1); active development of anti-CCR5-based therapies for HIV infection and acquired immune deficiency syndrome (AIDS) has shown promising results.^{14–16}

Based on the proinflammatory properties of CCR5⁺ T-cells and their role in immune activation, it was hypothesized that older individuals with frailty would have higher CCR5⁺ T-cell counts than nonfrail older persons. The purpose of this study was to test this hypothesis and to evaluate CCR5⁺ T-cells and their relationships with frailty in older adults. Addressing this hypothesis will improve understanding of potential underlying immune mechanisms that contribute to frailty and its associated chronic inflammation in older adults. It will also provide a basis for further phenotypic and functional characterization of CCR5⁺ T-cells and investigation into their role in the pathogenesis of frailty, with significant therapeutic implications.

MATERIALS AND METHODS

Human Subjects

Community-dwelling adults aged 72 and older from Baltimore, Maryland, were recruited from outpatient medical clinics, senior centers, and residential retirement communities and screened by a trained clinical coordinator. Frailty was ascertained using the validated screening criteria.¹ These criteria were based on the presence or absence of five measurable characteristics: weakness (grip strength), slowed motor performance (walking speed), fatigue, weight loss, and low levels of physical activity. Those meeting three or more of the five criteria were defined as frail and those meeting none as nonfrail.¹ Exclusion criteria included Parkinson's disease, cerebrovascular accident with residual hemiparesis, symptomatic congestive heart failure, active malignancy, uncompensated endocrine disorder, rheumatoid arthritis or any other inflammatory condition, significant cognitive deficit (Folstein Mini-Mental State Examination score less than 18/30), and usage of corticosteroids or other immune-modulating agents. These individuals were excluded to minimize the effect of a single disease in mimicking or initiating the presence of frailty or potential immune-modulating effects of medications. Those who qualified came to the General Clinical Research Center at Johns Hopkins Bayview Medical Center for a detailed history and physical examination by a physician investigator to ensure that they met the eligibility criteria and did not have an acute illness. The Johns Hopkins institutional review board approved the study protocol. Written informed consent was obtained from all participants.

Laboratory Studies

Fresh peripheral whole blood samples were collected, and the T-cell phenotypes were examined by immunofluorescence using different combinations of fluorescein isothiocyanate-, phycoerythrin-, allophycocyanin-, and peridinin-chlorophyll-protein complex-conjugated

monoclonal antibodies (mAbs) and appropriate negative isotype controls. Anti-CCR5 mAb clone 2D7/CCR5 was purchased from BD Bioscience (Mountain View, CA) and anti-CRTH2 from Miltenyi Biotec (Auburn, CA). All other mAbs were purchased from BD Bioscience or Caltag Laboratories (Invitrogen, Burlingame, CA). Red blood cells were lysed with an ammonium chloride-based solution (BD Pharm Lyse). To determine the percentage of T-lymphocytes (CD3⁺) in total lymphocytes and that of various T-cell subsets in the total T-cell pool, 100,000 events for each sample were recorded and analyzed on an FACSCalibur flow cytometer (Becton-Dickinson, San Jose, CA). Histogram analyses gated on lymphocytes (for total CD3⁺ T-cells) or CD3⁺ cells (for all T-cell subsets) were performed using FCS Express V3 software (De Novo Software, Thornhill, Ontario, Canada). To minimize potential laboratory variability, fresh whole blood samples from frail and nonfrail pairs were collected between 09:30 a.m. and 10:30 a.m. and flow cytometric analyses were performed within 1 to 2 hours and in parallel using the same reagents and equipment.

Peripheral blood lymphocyte counts as part of the complete blood counts were obtained from the same blood sample from each study participant using an automated (Coulter) counter at Quest Laboratory (Madison, NJ).

Data Analyses

Results are presented as means $\pm$ standard deviations. Statistical analyses were performed using Stata 9 (StataCorp, College Station, TX). The Wilcoxon signed-ranks test was used to evaluate differences in the study variables between frail and nonfrail participants using a two-sided α -value of .05 to determine statistical significance.

RESULTS

Demographic and Clinical Characteristics of the Study Participants

A total of 26 (13 pairs) community-dwelling frail and matched nonfrail participants completed the study. Table 1 summarizes basic demographic and clinical characteristics of the study participants. Because the frail and nonfrail study groups were age-, race-, and sex-matched, the two study groups had similar ages and the same race and sex composition, with a mean age of 83.8 ± 5.3 (range 72–94) and the majority being Caucasians and female. None of the participants were current or recent (within the previous 10 years) smokers. Both groups had comparable numbers of medical diagnoses and similar disease profiles. In addition, both groups were comparable in their total and specific medication use. None of the participants reported illicit drug use or heavy alcohol consumption.

Percentages of CCR5⁺ and Other T-Cell Phenotypes

As shown in Table 2, frail and matched nonfrail participants had similar percentages of total lymphocytes, T-lymphocytes, and CD45RO⁺ or CD45RO[−] T-cells, but frail participants had higher percentages of CD8⁺T-cells and lower percentages of CD4⁺T-cells than matched nonfrail controls. Furthermore, frail participants had significantly higher percentages of CCR5⁺ T-cells than matched nonfrail controls. No significant difference was observed between the two study groups in the frequencies of T-lymphocytes expressing CRTH2 ($4.9 \pm 4.4\%$ (frail) vs $4.8 \pm 3.5\%$ (nonfrail), $P = .93$), a reliable surface marker for human type-2 T-cells.

The phenotypes of CCR5⁺T-cells were further examined by evaluating additional T-cell surface markers. As shown in Table 2, frail participants had significantly higher percentages of CCR5⁺CD8⁺ and CCR5⁺CD45RO[−] T-cells than matched nonfrail controls. No significant differences were found between the two study groups in CCR5⁺CD4⁺ or CCR5⁺CD45RO⁺ T-cells.

Absolute Counts of CCR5⁺ and Other T-Cell Phenotypes

To further evaluate these findings, absolute counts of CCR5⁺ T-cells and other T-cell phenotypes were obtained from total peripheral lymphocyte counts determined in the same blood samples from each study participant. As shown in Table 2, no significant differences in the counts of peripheral lymphocytes or total T-cells were observed between the two groups. Of the major T-cell phenotypes examined using single surface markers, frail participants had significantly higher CD8⁺ and CCR5⁺ T-cell counts than nonfrail controls. Although the percentage of CD4⁺ T-cells was significantly lower in the frail group than in the nonfrail group (Table 2), this difference was no longer observed when determining absolute counts of CD4⁺ T-cells.

Examining CCR5⁺ T-cells according to double surface staining revealed that frail participants had significantly higher numbers of CCR5⁺CD8⁺ and CCR5⁺CD45RO⁺ T-cells than matched nonfrail controls. No significant differences in the counts of CCR5⁺CD4⁺ or CCR5⁺CD45RO⁺ T-cells were found between the two groups. To assess whether the higher CCR5⁺CD8⁺ T-cell counts were solely due to more CD8⁺T-lymphocytes, the frequencies of CCR5⁺T-cells in the CD3⁺CD8⁺ subset upon gating were evaluated according to the CD3⁺CD8⁺ cell population. Frail participants had higher percentages of CCR5⁺ T-cells than matched non-frail controls in the CD3⁺CD8⁺ T-cell population (65.8% ± 19.5% vs 55.6% ± 8.8%, $P = .08$).

Counts of CCR5⁺, CCR5⁺CD8⁺, and CCR5⁺CD45RO⁺ T-Cells Across Frailty Scores

Because frailty status was determined according to frailty measurement scores ranging from 3 to 5, counts of CCR5⁺, CCR5⁺CD8⁺, and CCR5⁺CD45RO⁺ T-cells were next examined across frailty scores. Six frail participants had a frailty score of 3, five had a score of 4, and two had a score of 5. As shown in Figure 1, there was a trend toward higher T-cell counts with greater frailty scores in frail participants. The differences between the groups did not reach statistical significance, probably because of the small number of subjects in each group.

DISCUSSION

The novel and significant initial findings of this study include that community-dwelling frail older adults had higher total CCR5⁺, CCR5⁺CD8⁺, and CCR5⁺CD45RO⁺ T-cell counts than age-, race-, and sex-matched nonfrail controls. With careful control of major demographic variables and comparable clinical profiles between the two study groups, the observed expansion of the CCR5⁺ T-cell population appears to be specific in frailty, above and beyond age-related T-cell remodeling, although these initial findings will need to be confirmed in large prospective studies.

Aging is associated with significant skewing of the immune repertoire to the memory phenotype.^{18,19} Consistent with this, it was observed that more than 50% of the total T-cell pool had a CD45RO⁺memory phenotype in frail and nonfrail participants. No differences were observed between the two study groups in CD45RO⁺ memory T-cell counts. In contrast, it was observed that frail older adults had higher CD8⁺ T-cell counts than matched nonfrail controls. This is consistent with the findings from a recent study that frailty is associated with higher frequencies of CD8⁺ T-lymphocytes in older women.²⁰

CCR5 is expressed on T-lymphocytes, monocytes, dendritic cells, and macrophages.^{8,9} Greater CCR5 gene expression in T-cells during aging has been demonstrated in humans and rodents at the messenger ribonucleic acid level and according to Western blotting,^{21,22} although the functional implication of this finding is unknown. Greater expression of chemokines and chemokine receptors, including CCR5, has been implicated in Alzheimer's

disease and other neurodegenerative diseases in which significant neuroinflammation is present.^{23,24} Although no significant differences in peripheral lymphocyte or total T-lymphocyte counts were observed between frail and nonfrail participants, the present study found that frail participants had higher percentages and absolute counts of total CCR5⁺, CCR5⁺CD8⁺, and CCR5⁺CD45RO⁺ T-cells than matched nonfrail controls, suggesting, for the first time, significant expansion of a specific T-cell subset with a type-1 proinflammatory phenotype in frail older adults. The observed trend of graded increase in the CCR5⁺ T-cell counts across frailty scores in frail participants (Figure 1), although preliminary, further suggests that CCR5⁺ T-cell counts may be correlated with frailty severity. The current evidence suggesting chronic inflammation as a key pathophysiological factor in frailty supports these observations. It may be that CCR5⁺ T-cells play an important role in the pathogenesis of frailty through their potential contribution to chronic inflammation in older adults, in addition to age-related immune remodeling.

These findings, if validated, have significant clinical and therapeutic implications. Anti-CCR5-based interventional strategies, with monoclonal antibodies or specific antagonists, have been highly successful in the treatment of HIV infection and AIDS in animal models and clinical trials.^{15,16} It is tempting to speculate that anti-CCR5-based strategies can be developed for the prevention or delay and treatment of the frailty syndrome in older adults.

No definitive conclusions can be drawn from this study because of its cross-sectional design with a small sample size. Nevertheless, these initial findings support the hypothesis and open a new and potentially important research avenue for better understanding of the pathogenesis of frailty. They also provide a basis for further evaluation of the CCR5⁺ T-cell subpopulation and its role in the development of frailty in older adults.

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References

1. Fried LP, Tangen C, Walston J, et al. Frailty in older adults: Evidence for a phenotype. *J Gerontol A Biol Sci Med Sci* 2001;56A:M1–M11.
2. Fried LP, Hadley EC, Walston JD, et al. From bedside to bench: Research agenda for frailty. *Sci Aging Knowledge Environ* 2005;2005:pe24. [PubMed: 16079413]
3. Lipsitz LA. Dynamics of stability: The physiologic basis of functional health and frailty. *J Gerontol A Biol Sci Med Sci* 2002;57A:115–125.
4. Leng S, Chaves P, Koenig K, et al. Serum interleukin-6 and hemoglobin as physiological correlates in the geriatric syndrome of frailty: A pilot study. *J Am Geriatr Soc* 2002;50:1268–1271. [PubMed: 12133023]
5. Walston J, McBurnie MA, Newman A, et al. Frailty and activation of the inflammation and coagulation systems with and without clinical morbidities: Results from the Cardiovascular Health Study. *Arch Intern Med* 2002;162:2333–2341. [PubMed: 12418947]
6. Leng SX, Xue QL, Tian J, et al. Inflammation and frailty in older women. *J Am Geriatr Soc* 2007;55:864–871. [PubMed: 17537086]
7. Leng SX, Cappola AR, Andersen RE, et al. Serum levels of insulin-like growth factor-I (IGF-I) and dehydroepiandrosterone sulfate (DHEA-S), and their relationships with serum interleukin-6, in the geriatric syndrome of frailty. *Aging Clin Exp Res* 2004;16:153–157. [PubMed: 15195991]

8. Mueller A, Strange PG. The chemokine receptor, CCR5. *Int J Biochem Cell Biol* 2004;36:35–38. [PubMed: 14592532]
9. Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med* 2006;354:610–621. [PubMed: 16467548]
10. Loetscher P, Uguccioni M, Bordoli L, et al. CCR5 is characteristic of Th1 lymphocytes. *Nature* 1998;391:344–345. [PubMed: 9450746]
11. Qin S, Rottman JB, Myers P, et al. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. *J Clin Invest* 1998;101:746–754. [PubMed: 9466968]
12. Locati M, Bonecchi R, Corsi MM. Chemokines and their receptors: Roles in specific clinical conditions and measurement in the clinical laboratory. *Am J Clin Pathol* 2005;123 Suppl:S82–S95. [PubMed: 16100870]
13. Gerard C, Rollins BJ. Chemokines and disease. *Nat Immunol* 2001;2:108–115. [PubMed: 11175802]
14. Liu R, Paxton WA, Choe S, et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 1996;86:367–377. [PubMed: 8756719]
15. Deeks SG. Challenges of developing R5 inhibitors in antiretroviral naive HIV-infected patients. *Lancet* 2006;367:711–713. [PubMed: 16517257]
16. Fatkenheuer G, Pozniak AL, Johnson MA, et al. Efficacy of short-term monotherapy with maraviroc, a new CCR5 antagonist, in patients infected with HIV-1. *Nat Med* 2005;11:1170–1172. [PubMed: 16205738]
17. Cosmi L, Annunziato F, Galli MIG, et al. CCR2 is the most reliable marker for the detection of circulating human type 2 Th and type 2 T cytotoxic cells in health and disease. *Eur J Immunol* 2000;30:2972–2979. [PubMed: 11069080]
18. Miller RA. The aging immune system: Primer and prospectus. *Science* 1996;273:70–74. [PubMed: 8658199]
19. Pawelec G, Barnett Y, Forsey R, et al. T cells and aging, January 2002 update. *Front Biosci* 2002;7:d1056–d1183. [PubMed: 11991846]
20. Semba RD, Margolick JB, Leng S, et al. T cell subsets and mortality in older community-dwelling women. *Exp Gerontol* 2005;40:81–87. [PubMed: 15664735]
21. Chen J, Mo R, Lescure PA, et al. Aging is associated with increased T-cell chemokine expression in C57BL/6 mice. *J Gerontol A Biol Sci Med Sci* 2003;58A:975–983. [PubMed: 14630877]
22. Yung RL, Mo R. Aging is associated with increased human T cell CC chemokine receptor gene expression. *J Interferon Cytokine Res* 2003;23:575–582. [PubMed: 14585197]
23. Cartier L, Hartley O, Dubois-Dauphin M, et al. Chemokine receptors in the central nervous system: Role in brain inflammation and neurodegenerative diseases. *Brain Res Brain Res Rev* 2005;48:16–42. [PubMed: 15708626]
24. Balistreri CR, Caruso C, Grimaldi MP, et al. CCR5 receptor: Biologic and genetic implications in age-related diseases. *Ann NY Acad Sci* 2007;1100:162–172. [PubMed: 17460174]

Figure 1.

Trend of graded increase in CCR5⁺ T-cell counts across frailty scores. Each bar represents the mean count of CCR5⁺T-cells (A), CCR5⁺CD8⁺ T-cells (B), and CCR5⁺CD45RO⁻ T-cells (C) in each of the frail participant groups with a frailty score of 3, 4, or 5. The numeric data of mean $\pm$ standard deviation are shown above each bar. The number of frail participants in each group is indicated (in parenthesis) along with the frailty score.

Table 1
Demographic and Clinical Characteristics of the Study Participants

Characteristic	Frail (n = 513)	Nonfrail (n = 513)	P- Value
Age, mean $\pm$ SD (range)	83.8 $\pm$ 5.3 (72–94)		
White, %	92.3		
Female, %	84.6		
Body mass index, kg/m ² , mean $\pm$ SD	26.8 $\pm$ 5.5	27.2 $\pm$ 4.8	.37
Comorbid chronic conditions			
Number of diagnoses, mean $\pm$ SD	4.5 $\pm$ 1.8	4.2 $\pm$ 1.8	.73
Specific diseases, %			
Hypertension	53.9	76.9	
Osteoarthritis	53.9	46.2	
Congestive heart failure (well controlled)	38.5	30.8	
Coronary heart disease (asymptomatic)	15.4	30.8	
Hypercholesteremia	23.1	38.5	
Malignancy (reported cured)	15.4	38.5	
Osteoporosis	30.8	23.1	
Hypothyroidism (compensated)	30.8	23.1	
Medication usage			
Number of medications, mean $\pm$ SD	4.6 $\pm$ 1.9	4.2 $\pm$ 2.0	.47
Specific medications, %			
Aspirin	30.8	30.8	
β -blocker	38.5	46.2	
Diuretic	30.8	38.5	
Calcium-channel blocker	46.2	30.8	
Angiotensin-converting enzyme inhibitor	30.8	23.1	
3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor	23.1	30.8	

SD = standard deviation.

Table 2
Total T-Lymphocytes and Specific T-Cell Subpopulations in Frail and Nonfrail Participants

Study Variable	Percentages		Absolute Numbers/mm ³		P-Value
	Frail (n = 13)	Nonfrail (n = 13)	Frail (n = 13)	Nonfrail (n = 13)	
	Mean ± SD		Mean ± SD		
Total lymphocytes	20.4 ± 8.5	25.1 ± 8.1	1,628 ± 531	1,357 ± 467	.18
T-lymphocytes (CD3 ⁺) (gated on lymphocyte)	67.3 ± 9.4	67.2 ± 10.3	1,092 ± 336	917 ± 364	.24
T-cell subsets (gated on CD3 ⁺)					
Single marker					
CD4 ⁺	70.7 ± 9.7	78.9 ± 6.9	773 ± 267	721 ± 294	.67
CD8 ⁺	29.2 ± 9.3	21.0 ± 7.2	319 ± 110	195 ± 97	.02
CD45RO ⁺	57.6 ± 14.7	54.8 ± 11.1	569 ± 264	470 ± 258	.41
CD45RO ⁻	42.4 ± 14.6	45.2 ± 11.1	425 ± 222	393 ± 230	.74
CCR5 ⁺	32.6 ± 12.7	20.6 ± 5.4	349 ± 160	194 ± 168	.02
Double markers					
CCR5 ⁺ CD4 ⁺	13.5 ± 7.5	9.8 ± 3.3	142 ± 75	93 ± 59	.15
CCR5 ⁺ CD8 ⁺	19.1 ± 6.8	11.6 ± 5.7	208 ± 98	105 ± 62	.02
CCR5 ⁺ CD45RO ⁺	18.8 ± 5.8	17.8 ± 6.4	198 ± 74	164 ± 100	.37
CCR5 ⁺ CD45RO ⁻	16.9 ± 11.2	5.6 ± 3.6	189 ± 149	52 ± 36	.01

SD = standard deviation; CCR5 = CC chemokine receptor-5.