Published in final edited form as:

Cancer Immunol Immunother. 2015 November ; 64(11): 1429–1435. doi:10.1007/s00262-015-1738-9.

The impact of leukapheresis on immune-cell number and function in patients with advanced cancer

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Abstract

Background—Leukapheresis is often performed in cancer patients to harvest stem cells, manufacture therapeutic vaccines, or follow immunologic response to therapy. We have recently described the minimal impact of leukapheresis on normal donors. Here we provide additional immunologic data from patients with advanced cancer who underwent leukapheresis.

Methods—Using data from cancer patients on clinical trials who had leukapheresis ($n = 64$) or peripheral blood draws only ($n = 90$) as controls for immune analysis, we evaluated the impact of leukapheresis on number and function of lymphocytes.

Results—In the leukapheresis group, median age was 63.5 (range 38–82); 87.5 % were male. Comparing pre- and post-leukapheresis values within the groups, with each patient as its own control, there was no significant difference in enzyme-linked immunosorbent spot (ELISPOT), antivector humoral response, absolute lymphocyte count (ALC), or T cell number. Twelve patients completed three leukaphereses with subsequent ELISPOT analysis; seven had increased responses to flu (1.1- to 2.3-fold) with an even distribution around no change. Nineteen patients had matched ALC values after completing three leukaphereses with no significant change from baseline.

Conclusions—These data provide evidence that leukapheresis has no detectable effects on a cancer patient's immune system in terms of number or function. These results contribute to a growing body of evidence refuting the hypothesis that a patient's immune competence is meaningfully affected by the procedure. Limitations include a restriction to 2-L leukapheresis procedure and small sample size.

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Compliance with Ethical Standards

Conflict of interest No potential conflicts of interest were disclosed.

Keywords

Apheresis; Vaccine; Immunotherapy; Clinical trial

Introduction

In 1981, the US Food and Drug Administration (FDA) issued guidance on leukocyte collection, stating that since there was a potential risk associated with the collection of large numbers of lymphocytes from a donor, it was not known how frequently such donations could be made. In 1988, the FDA issued further guidelines on apheresis, suggesting that the number of procedures should not exceed 24 per year. This guidance was updated in 2007 [1]. Professional societies have also issued guidelines limiting apheresis to no more than 2 per week or 24 per year [2, 3].

Leukapheresis removes lymphocytes within the blood. However, circulating lymphocytes represent only a fraction of the total number of lymphocytes in the human body (approximately 2 %) [4]. Furthermore, lymphocytes can readily traffic from the organs into the blood. While there are recognized risks of lymphodepletion in individuals losing over 1×10^{11} lymphocytes over a brief period, or with baseline lymphocyte counts of under $0.5 \times 10^9/\text{L}$ [5], a more limited 1.5–2 blood volume leukapheresis (about 7×10^9 lymphocytes) is not associated with any clinically significant immunodepletion. A large study of over 400 healthy volunteers (median age 34, range 17–70) who underwent 4957 leukapheresis procedures (mean volume processed 6.8 L, range 2–10) at the National Institutes of Health (NIH) has been presented [6, 7]. The vast majority of leukapheresis procedures completed (3370) were within 56 days of a previous leukapheresis (median 23 days). Patients undergoing serial leukapheresis at appropriate intervals had a clinically insignificant 3.6 % decrease in circulating absolute lymphocyte count (ALC) between leukapheresis time points. The estimated cumulative lymphocyte loss after 2–9 leukapheresis procedures was 9.7 % in the peripheral blood, which is <0.2 % of total body lymphocytes. Most importantly, history and physical exam data were reviewed and revealed no increased susceptibility to infectious diseases or cancer.

While this provides an excellent baseline dataset for the impact of leukapheresis in healthy young patients (median age was 34), investigating the effects of the procedure in advanced cancer patients would provide a more clinically relevant context. It would also explore a population in which apheresis may be more likely to have a negative immune impact, as cancer patients are already considered to have impaired immune function at baseline and are likely to be older, particularly in the case of prostate cancer. This may be important, as reports have suggested a gradual decrease in immune repertoire over time [8]. Indeed, the effects of aging on the immune system have been used to suggest that leukapheresis may actually be deleterious to older patients. In addition, the previous dataset only addressed a quantitative measure of blood-cell subsets without any assessment of function. To address these limitations, we explored data from 12 clinical studies (nine at NIH, three collaborations with Georgetown University) [9–20] in cancer patients who had immunologic

analysis before and after either a phlebotomy procedure or leukapheresis for evidence of the impact of leukapheresis on immune-cell subset number and function.

Methods

Patients and procedures

We evaluated 154 patients with solid tumors enrolled in IRB-approved studies [21–23] using experimental off-the-shelf immunotherapy approaches. Study details are available in Supplemental Table 1. All patients had a history, physical exam, and clinical laboratories prior to leukapheresis, and leukapheresis was only performed if there was no evidence of infection. All patients had immune analysis at baseline and approximately 3 months later using cells obtained via either leukapheresis ($n = 64$) or simple phlebotomy as a control (leukapheresis typically had a 2.0-L leukapheresis at baseline ($n = 90$). Patients who underwent leukapheresis and a second procedure about 85 days later, using intermittent flow centrifugation, with an average flow rate of 50–60 mL/min, depending on patient body size and venous access. Approximately 1.4×10^9 leukocytes per liter processed are typically harvested by such procedures; about 75 % of the cells in the apheresis product are lymphocytes, and about 25 % are monocytes [6]. The lymphocytes obtained are about 50–60 % T cells and 15–20 % B cells. Fifty-two patients underwent two and 12 patients underwent three leukapheresis procedures within the 85-day period of the studies.

Assays

To determine functional immune responses, the IFN- γ enzyme-linked immunosorbent spot (ELISPOT) was performed in our laboratory, which has extensive experience with this assay, as previously described [24]. ELISPOT is expressed as the number of antigen-specific T cells (producing IFN- γ in response to antigen) out of 1,000,000 peripheral blood mononuclear cells (PBMCs). In addition, cell numbers (ALC, CD3, CD4, and CD8) were obtained through the NIH Clinical Center Laboratory, a Clinical Laboratory Improvement Act (CLIA)—and College of American Pathologists (CAP)—certified clinical laboratory, using a Beckman Coulter instrument for lymphocyte phenotyping. Humoral responses [antivaccinia virus immunoglobulin G (IgG)] were detected by enzyme-linked immunosorbent assay (ELISA), as previously described [14]. All of the immunotherapy studies had defined prospective collections of PBMCs and serum for these assays, and raw data were used for the current analysis.

Statistical analysis

For all parameters, baseline (pre) values were compared to post-treatment (post) values using a Wilcoxon signed-rank test. Continuous parameters were compared between patients with and without leukapheresis using a form of an exact Wilcoxon rank-sum test. Fractions with positive assays (responses) were compared between the two groups using Fisher's exact test. p values are two-tailed and presented without any formal adjustment for the number of tests performed. However, in view of the number of comparisons made, only $p < 0.01$ should be interpreted as a statistically significant effect; $0.01 < p < 0.05$ would be considered a strong trend.

Results

Among patients who underwent leukapheresis ($n = 64$), the median age was 63.5 (range 38–82); 87.5 % were male. Additional baseline characteristics are found in Supplemental Table 2. All patients had adequate bone marrow function at baseline, as required for eligibility on the immunotherapy protocols. Details of the baseline hematologic parameters for the leukapheresis patients are available in Supplemental Table 3.

Impact of leukapheresis on cell counts

Of the 64 patients tested, 51 had paired samples of ALC pre- versus 7–36 days post-leukapheresis and 19 had immune-cell subsets analyzed 7–36 days after leukapheresis (Fig. 1a–d). There was no change in the proportion of T cells present in the blood (relative values; Fig. 1c) or in the composition of the T cell compartment with respect to the ratio of CD4 to CD8 T cells (Fig. 1d). There was no significant change in ALC, CD3⁺, CD4⁺, or CD8⁺ cells following leukapheresis in those patients, with a trend toward slightly increased numbers in all subsets. These trends persisted in samples taken approximately 85 days post-leukapheresis, with 26 paired ALC samples and 36 immune subsets (Fig. 1a–d). The only strong trends toward significant changes were increases in CD3⁺ and CD4⁺ numbers.

Impact of leukapheresis on T cell function

To examine the function of immune cells, we looked for evidence of the ability of flu-specific T cells to secrete IFN- γ . We tested PBMCs from patients who had either leukapheresis ($n = 64$) or simple phlebotomy ($n = 90$) (Table 1). There was no quantitative or qualitative decrease in the ability of flu-specific T cells to secrete IFN- γ in response to flu matrix protein with either serial blood draws or leukapheresis. ELISPOT responses were evaluated in 12 patients following a third leukapheresis. Responses to flu were generally unchanged following leukapheresis, with seven patients demonstrating improved responses relative to baseline (1.1- to 2.3-fold increases). Statistical analysis identified no consistently positive or negative changes in cell subsets in patients who underwent leukapheresis.

Impact of leukapheresis on humoral response

We also evaluated humoral responses to vaccinia ($n = 74$), which was given to all patients as the initial vaccine vector in the setting of therapeutic vaccine clinical trials (Table 2). There was no significant change in the proportion of responders ($p = 0.0237$) or the titer of response ($p = 0.0179$) in patients who underwent leukapheresis compared with those who underwent simple phlebotomy, though there was a trend toward increased responses in the leukapheresis group. This trend is likely a function of small patient numbers, but the lack of a decrease or a significant difference suggests that there was no functional impairment of B cell responses as a result of leukapheresis.

Impact of age on immunity following leukapheresis

If leukapheresis were disproportionately harmful to older subjects, one might expect to observe a post-procedure deterioration in laboratory measurements of immune parameters that increase with age. To determine the impact of leukapheresis on immune function, flu-specific T cells were quantified using ELISPOT. A comparison of pre- and post-

leukapheresis values within the groups, with each patient as their own control, revealed no significant difference by ELISPOT (Table 3). Importantly, when ELISPOT responses were examined in subjects above and below the median age or by age quartiles, no significant changes were observed, nor were changes significant when age was considered as a continuous variable.

Discussion

In 2010, sipuleucel-T became the first therapeutic cancer vaccine to be approved by the FDA, based on a 4.1month improvement in median overall survival (HR 0.77, $p = 0.02$) in men with metastatic castration-resistant prostate cancer receiving sipuleucel-T versus control infusions. It has been suggested that leukapheresis, used to produce the vaccine sipuleucel-T, may negatively affect the overall survival of cancer patients by removing circulating lymphocytes and diminishing host immunity, especially in the control arm where patients receive only 1/3 of the harvested cells back, with the remainder stored for subsequent vaccine manufacture at crossover [25]. In the published subgroup analysis, all subgroups, including those above and below the median age, showed evidence of benefit from sipuleucel-T versus control. However, a separate subgroup analysis performed to meet Center for Medicare and Medicaid Services requirements showed that in a minority of patients less than 65 years old, there appeared to be no difference in overall survival. However, it should be noted that only 66 control patients (9.0 %) were under the age of 65 out of 737 total patients analyzed and that the inherent risk of a subset analysis is further magnified by the small number of patients in this subset. Huber et al. hypothesized that the difference in overall survival seen in the trial as a whole and in the men over 65 was due to a greater removal of immune cells in the control arm, causing immune suppression that was magnified in older men [25].

In addition to other academic arguments against the immune depletion hypothesis [26, 27], the difference in the proportion of total body lymphocytes removed between control patients and patients treated with sipuleucel-T is <1 %, with no evidence of increased infections [28]. In addition, patients getting active product were able to mount significant immune responses that correlated with overall survival [29].

Our analysis suggests that a 2-L leukapheresis has no measurable impact on the number or function of T cells and no negative impact on the ability to mount a humoral immune response to vector. It should also be noted that leukapheresis (baseline) immediately preceded vaccine (typically on the same day), further suggesting that the procedure did not interfere with patients' ability to mount a de novo immune response. Furthermore, there is no suggestion of an age-based differential effect post-leukapheresis on measured immune outcomes.

This study had several limitations. First, we only had data from 154 patients, 64 of whom underwent leukaphereses, treated on 12 different immunotherapy trials. Although raw data were analyzed for the purposes of this study, not all analyses were performed for all patients due to several factors, including HLA status (for ELISPOT analysis), lack of availability of post-leukapheresis samples at comparable time points, and variations in protocol design.

However, all patients served as their own controls, mitigating the potential impact of the variability between protocols. It is possible that small differences would emerge in an analysis of larger numbers of patients. It should also be noted that the leukaphereses in this study were 2-L procedures, rather than the 1.5–2.0 times the patient's estimated blood volume (approximately 7–10 L) used in the sipuleucel-T study. Furthermore, the leukaphereses in our study were typically performed 2–3 months apart, which differs from the biweekly schedule for three procedures used in the manufacture of sipuleucel-T. However, in terms of immune depletion, while our 2-L leukapheresis procedures resulted in less estimated loss of peripheral leukocytes (3×10^9), none of these cells were reinfused into patients, as they were in the sipuleucel-T studies, so the overall impact is likely similar.

Our analysis found no discernible decline in the number or function of immune cells in cancer patients undergoing small-volume leukapheresis. Thus, the hypothesis that immune competence is significantly compromised by the procedure is inconsistent with available data. However, in patients with bone marrow insufficiency or those undergoing larger-volume or more frequent leukapheresis procedures, further studies would need to be done to rule out any significant change in immune parameters following leukapheresis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge the Intramural Research Program of the National Institutes of Health (NIH), National Cancer Institute (NCI), Center for Cancer Research for their support of this study. We also express appreciation to the professionals at the NIH Clinical Center Department of Transfusion Medicine for their part in performing apheresis procedures in study patients and to the medical oncology fellows at the NCI for their attention to patient care. Finally, we thank Bonnie L. Casey and Debra Weingarten for their editorial assistance in the preparation of this manuscript. This research was supported by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, National Institutes of Health.

Abbreviations

ALC	Absolute lymphocyte count
CAP	College of American Pathologists
CLIA	Clinical Laboratory Improvement Act
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunosorbent spot
FDA	US Food and Drug Administration
IFN-γ	Interferon-gamma
IgG	Immunoglobulin G
NIH	National Institutes of Health

OS	Overall survival
PBMCs	Peripheral blood mononuclear cells

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Fig. 1.
a–d Impact of 2-L leukapheresis on immune-cell subsets in cancer patients, comparing baseline to 1 month post-leukapheresis (day 7–36) and baseline to approximately day 85 post-leukapheresis. In all panels, *p* values are the result of a paired Wilcoxon signed-rank test of the difference between pre-treatment values and values at 1 month or day 85. **a** Impact of leukapheresis on absolute lymphocyte counts. **b** Impact of leukapheresis on CD3, CD4, and CD8 subsets, expressed in cells/ μ L of whole blood. **c** Impact of leukapheresis on CD3, CD4, and CD8 subsets, expressed as percentage of peripheral blood lymphocytes. **d** Impact of leukapheresis on CD4/CD8 ratio

Table 1

Impact of leukapheresis versus simple peripheral blood collection on the proportion of flu-specific T cells identified via IFN- γ ELISPOT in cancer patients (number of antigen-specific cells per 10^6 PBMCs), comparing baseline values with those at approximately day 85 post-leukapheresis

	<u>Leukapheresis group $n = 64$</u>		<u>Nonleukapheresis group $n = 90$</u>	
	Baseline	Day 85	Baseline	Day 85
Mean	77.43	74.05	47.99	51.30
Median	45.83	48.33	30.00	29.17
Minimum-maximum	5.00-503.27	5.00-794.28	5.00-495.05	5.00-416.67
p value: absolute difference pre-/post-within groups	0.83		0.76	
p value: absolute difference pre-/post-between groups	0.85			
p value: ratio pre-/post-within groups	0.35		0.32	
p value: ratio pre-/post-between groups	0.94			

p values within groups calculated using two-tailed Wilcoxon signed-rank test. p values for comparisons between groups calculated using an exact two-tailed Wilcoxon rank-sum test

Impact of leukapheresis versus simple peripheral blood collection on the proportion of post-vaccine viral vector-specific antibody responses in cancer patients

Table 2

	Leukapheresis group	Nonleukapheresis group
Proportion of subjects with positive assay—# positive/total (%)	30/33 (90.91)	28/41 (68.29)
Median titer post-vaccinia	1/5000	1/1100
Range of titer post-vaccinia	1/50->1/>6250	1/<50->1/>8000
Median fold change in titer	8.33	5.00
p value for proportion of responders	0.0237	
p value: fold change from baseline	0.0179	

p value for comparison of proportion of responders between two leukapheresis groups calculated using Fisher's exact test; p value for comparing fold changes calculated using Wilcoxon rank-sum test

Impact of leukapheresis versus simple peripheral blood collection on ratio of flu-specific T cell pre-treatment to approximately day 85 by age quartile of subject ($n = 154$)

Table 3

Age quartile	20–54		55–63		64–69		70–87	
	No	Yes	No	Yes	No	Yes	No	Yes
<i>N</i>	26	14	26	18	19	14	19	18
Mean	1.08	1.53	1.15	0.98	0.96	1.38	1.17	1.76
Median	1.00	1.00	1.00	0.93	0.90	1.26	1.06	1.01
Range	0.21–2.10	0.50–4.80	0.35–2.95	0.21–1.95	0.46–2.09	0.22–3.25	0.50–2.81	0.21–12.5
<i>p</i> value: relative change, ratio	0.86		0.43		0.20		0.52	