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The effect of pelvic radiation alone on lymphocyte subgroups

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ABSTRACT

There is a lack of information on the radiosensitivity of lymphocyte subgroups to radiation alone. CD4+ and CD8+ lymphocytes respond similarly. CD 19+ dropped most precipitously, but recovered to levels similar to the other subgroups by 3 months. NK cells decline more modestly and recover more fully by 3 months.

1. Introduction

Lymphocytes are considered the mainstay of anticancer immunity. As a result, there is significant interest in defining and enhancing their role. While the benefits of radiation therapy are well established, the interplay of radiation and immunity are less well understood. Lymphocytes are considered one of the most radiosensitive mammalian cells. The initial concerns for the impact of immune suppression have more recently been balanced by evidence that radiation therapy may enhance cellular immunity. There is much to be learned about those interactions, especially regarding lymphocyte subgroups. Almost all the studies of the effect of radiation on the various subgroups contain chemotherapy, which has its own profound effects on lymphocytes. To better understand the direct effect of radiation therapy on lymphocytes, we collected data in a well-defined patient group with fairly uniform radiation treatment without the confounding effect of systemic therapy.

2. Materials and methods

With institutional review board (IRB) approval and with informed consent, a prospective study was undertaken in consecutive male patients receiving radiation for prostate cancer. One patient was found to have metastatic disease shortly after consent and was excluded, but a total of 15 patients were recruited. No patient received chemo or hormonal therapy. All patients had the pelvic lymphatics treated to 54 Gy. The lymphatics were defined by the major blood vessels, including the internal and external iliac up to the common iliac vessels. The volume of the pelvis (including the pelvis proper, the proximal femurs to the level of this ischium, the sacrum and L4/5) was drawn and the volume receiving >20 Gy (well above the known sensitivity of lymphocytes) was calculated.

After the initial pelvic radiation, most of the patients (n = 11) received 70 Gy to the prostate fossa, 3 received 78 Gy to the prostate and one underwent a brachytherapy boost. Blood for complete blood counts (CBC) and flow cytometry were collected right before the start of treatment (within 2 weeks), end of pelvis treatment (within a week) and 3 months post therapy. (Table 1 for characteristics). Cluster of differentiation (CD) markers were used to determine lymphocyte subgroups via flow cytometry. CD3+ cells are considered T lymphocytes, with the CD3+ subgroups of CD4+ (T helper) and CD8+ (T cytotoxic). CD 19+ are considered B cells and CD56+ are natural killer (NK) cells. For brevity, going forward, the CD3+ CD4+ and CD3+ CD8+ will be referred to as CD4+ and CD8+, respectively. Testing was accomplished with the three color TriTest monocolonal antibody panel for CD3/CD8/CD45, CD3/CD4/CD45, CD3/CD19/CD45 and CD3/CD16+ CD56/CD45 with analysis on the Becton Dickinson Macintosh FACSCalibur system (Becton Dickinson and Company, Franklin Lakes NJ, USA). Each specific reagent (20 μL) is added to separate tubes containing 50 μL of whole blood and vortexed and incubated at room temperature for 15–30 min. Then 0.5 ml of BD Lyse Solution is added and incubated for 15 min. The specimens are then loaded on the flow cytometry analyzer and run as per standard and data collected.

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Table 1
Baseline patient characteristics.

	Mean/median	range	Standard deviation
Age (years)	64/64	52–79	
Total lymphocyte/μL	1998/1922	1113–3192	617
CD3+/μL	1378/1388	603–246	524
CD4+/μL	856/924	298–1319	309
CD8+/μL	501/428	190–1280	283
CD19+/μL	318/241	130–1054	231
CD56+/μL	255/192	111–753	169

Table 3
Percent of each subgroup as a constituent of the total lymphocyte population. Note that CD3+ includes both CD3+ CD4+ and CD3+ CD8+ lymphocytes.

	Baseline % Mean/median	End of treatment % mean/median	3 Months % mean/median
CD3+	69%/69%	71%/72%	63%/61%
CD4+	43%/43%	45%/48%	36%/37%
CD8+	24%/25%	25%/24%	25%/22%
CD19+	16%/14%	6%/4%	15%/14%
CD56+	13%/12%	18%/21%	19/17%

3. Statistics

Sample characteristics were described using descriptive statistics. Frequencies and percentages were used to describe categorical variables. Means and standard deviations (or medians and ranges where appropriate) were used to describe continuous variables. A one-sample *t*-test (or Wilcoxon signed-rank test when appropriate) was used to test if specific lymphocyte components experiences a significant change over time. A two-sample *t*-test (or Wilcoxon rank-sum test when appropriate) was used to test for differences in lymphocyte counts between dose groups. A generalized estimating equation (GEE) was used to assess the difference each specific lymphocyte component experienced between time points, while adjusting for intra-patient correlations. Post-hoc Tukey-Kramer adjustments were made for multiple comparisons within each GEE model. Statistical significance was set to $p < 0.05$.

4. Results

An average of 61% (range 53–69%) of the pelvic bone volume receiving ≥20 Gy. There was no difference between whether the patient was post prostatectomy versus intact prostate in the degree of lymphocyte decline.

The overall leukocyte (white blood cell [WBC] count) declined from a mean of 6370 cells/μL to 4070 cells/μL (34% decline) by the end of treatment, and remained relatively stable (4510 cells/μL) at 3 months, primarily due to the slow recovery of the lymphocytes. (Table 2).

Overall, the total lymphocyte count declined by 73% at the end of treatment (to 27% of baseline) (Table 2). At 3 months, the lymphocytes had recovered, but were still <50% of the starting value. The CD3+, CD4+, CD8+, and CD56+ lymphocyte subgroups were equally sensitive when compared to each other. The CD19+ was significantly more sensitive, declining to just 9% of initial levels. While not significantly different ($p > 0.1$), the CD56+ cells showed a trend to being more resistant, with less of a decline (about 10% less) than the CD3+, CD4+ and CD8+ subgroups. None of the subgroups were back to normal levels at 3 months, but the recovery of the CD56+ cells ($P < 0.0001$) was more complete than the other groups. Also, although they were more sensitive, the recovery of the CD19+ cells was more robust ($P < 0.0001$) than the other subgroups, so that there was no difference in the per cent decline from baseline at 3 months compared to the other subgroups.

As a percentage of the total lymphocytes, because of their greater sensitivity, at the end of treatment the per cent of CD19+ cells had declined and, with their relative resistance, the percent of CD56% cells increased. (Table 3) At three months as cells recovered, the per cent contribution of CD4+ cells had declined some from the end of treatment and the CD56+ increased.

5. Discussion

Lymphocytes are extremely sensitive to radiation. In our patients, the total lymphocyte count dropped 73%. At 3 months, recovery occurs, but is incomplete. We showed that as a whole the CD3+ subgroup and specifically the CD3+ CD4+, the CD3+ CD8+ and the CD56+ subgroups exhibited the same radiosensitivity. CD19+ cells were statistically more radiosensitive, but recovered more rapidly so that at 3 months the overall decline was no different than the other subgroups. NK (CD56+) cells were somewhat more resistant and at 3 months exhibited a statistically significant smaller decline from baseline than the other lymphocyte sub types. Overall, at the end of treatment, CD4+ and CD8+ had declined about 72% and at 3 months were about 60% below baseline. CD19+ declined 91%, but at 3 months was about 60% below baseline and CD56+ cells initially declined 62% but were only 36% below baseline at 3 months (Table 2).

There are few studies that have evaluated the effect of radiation therapy alone on lymphocyte subgroups and reported the actual lymphocyte counts (Table 4). Two were in cervix cancer. The first (1) reported on 39 cervix cancer patients with radiation alone. From the figures, CD8+ counts dropped from a mean of 670 cells/μL to 280 cells/μL (59% decline) and CD56+ from 350 cells/μL to 190 cells/μL (46% decline). They did not measure CD4 cells. As a percentage of the total lymphocyte count, CD8+ count increased from 27% to 32% and the CD56 from 13% to 20%. They did not report recovery data. In the second study (2) there were 14 patients with pelvic radiation alone and the total lymphocyte count declined from a mean of 1137 cells/μL to 357 cells/μL (69% decline), which improved to 639 cells/μL (overall 44% decline) by 6 months (a 25% recovery). For each of the subgroups, CD4+ declined from 566 cells/μL to 202 cells/μL (64% decline) and at 6 months 223 cells/μL (61% decline overall, 3% improvement from end of treatment); CD8+ from 323 cells/μL to 196 cells/μL (39% decline), at 6 months 298 cells/μL (8% decline, 31% recovery); CD19+ from 132 cells/μL to 27 cells/μL (80% decline), at 6 months

Table 2
Change in blood counts from pretreatment to end of treatment and at 3 months post treatment.

	Mean/medianbase	Mean/median end	% Decline from base	Mean/median 3 months	% Decline from base
Total leukocytes	6370/6100	4070/4200	34%/34%	4510/4500	28%/31%
Total lymphocytes	1998/1922	524/470	73%/73%	832/848	57%/56%
CD3+	1378/1388	383/367	72%/75%	531/467	60%/62%
CD4+	856/924	236/211	73%/75%	305/294	63%/65%
CD8+	501/428	138/130	72%/76%	159/270	57%/58%
CD19+	318/241	25/20	91%/91%	113/93	59%/62%
Cd56+	255/192	95/67	62%/68%	155/121	36%/36%

Table 4
Comparative data.

	Total lymphocyte (μL)					CD3+ (μL)				
	Current	Eric [1]	Bach-tiary [2]	Belka [3]	Maehata [4]	Current	Eric [1]	Bach-tiary [2]	Belka [3]	Maehata [4]
Base	2998		1137	1405	1363	1378		872	952	872
End	524		357	462	917	383		374	323	636
Decline	73%		69%	67%	33%	72%		67%	66%	27%
f/u	3 mos		6 mos	4 mos		3 mos		6 mos	4 mos	
	832		639	858		531		484	593	
Decline	57%		44%	39%		60%		44%	38%	
	CD4+ (μL)					CD8+ (μL)				
	Current	Eric	Bach-tiary	Belka	Maehta	Current	Eric	Bach-tiary	Belka	Maehata
Base	856		566	560	566	501	670	232	421	299
End	236		202	202	424	138	280	196	130	209
Decline	73%		64%	64%	25%	72%	59%	39%	69%	30%
f/u	3 mos		6 mos	4 mos		3 mos		6 mos	4 mos	
	305		223	333		159		298	260	
Decline	63%		61%	41%		57%		8%	38%	
	CD19+ (μL)					CD56+ (μL)				
	Current	Eric	Bach-tiary	Belka	Maehta	Current	Eric	Bach-tiary	Belka	Maehata
Base	318		132	153	163	255	350	251	116	306
End	25		27	14	80	95	190	129	29	188
Decline	91%		80%	91%	51%	62%	46%	49%	75%	49%
f/u	3 mos		6 mos	4 mos		3 mos		6 mos	4 mos	
	113		88	64		155		222	102	
Decline	59%		33%	58%		36%		12%	12%	

88 cells/μL (33% decline, 47% improvement) and CD56+ from 251 cells/μL to 129 cells/μL (49% decline, at 6 months 222 cells/μL (12% decline, 37% improvement). Both of these studies had patients with whole pelvis treatment, so volumes were similar to ours. There was a big difference in the baseline counts, probably reflecting the different patient populations, labs and methodology, but the cell count declines and recovery were similar.

There were two additional studies with dissimilar treatment volumes. The first consisted of 10 seminoma patients given 26 Gy to the para aortic lymphatics (3). They noted no differences in the baseline counts of the patients and healthy volunteers. The total lymphocyte count dropped from 1405 cells/μL to 562 cells/μL at the end of treatment (67% decline) and was 858 cells/μL (39% decline, 28% improvement) at 4 months. For CD4+ cells baseline was 560 cells/μL, declining to 202 cells/μL (64% decline) and 333 cells/μL at 4 months (41% decline, 23% improvement); CD8+ 421 cells/μL, to 130 cells/μL (69% decline) and 260 cells/μL at 4 months (38% decline, 31% improvement); CD19+ 153 cells/μL to 14 cells/μL (91% decline) and 64 cells/μL at 4 months (58% decline, 33% improvement): and CD56+ 116 cells/μL to 29 cells/μL (75% decline) and 102 cells/μL at 4 months (12% decline, 63% improvement).

In the second study, 36 lung cancer patients (4) were treated with focal (small field) therapy with either 40–60 Gy in 4 fraction or 60–70 Gy in 10 fractions. The total lymphocyte count declined from a mean of 1363 cells/μL to 927 cells/μL (69% decline); CD4+ declined from 566 cells/μL to 424 cells/μL (64% decline); CD8+ from 299 cells/μL to 209 cells/μL (39% decline); CD19+ from 162 cells/μL to 80 cells/μL (80% decline), and CD56+ from 306 cells/μL to 188 cells/μL (49% decline). They did not report follow up levels. In general, with the smaller volumes they used, when compared to ours and the other studies, the decline was not as profound, except in the B cell (CD 19+) population. This would suggest that since we are well above the dose sensitivity of lymphocytes that volume treated becomes more important. A study using stereotactic body radiation therapy (SBRT) to small volumes in pancreatic cancer confirmed a more modest decline (35%) in total lymphocyte counts [5] than we see with larger field therapy.

In spite of the disparities in the populations, what is apparent is that $CD3^+$ and the $CD3^+$ $CD4^+$ subgroups have similar radiosensitivity. $CD3^+CD8^+$ is also similar, but more variable between the studies. $CD19^+$ cell are the most radiosensitive, but recover more quickly. $CD56^+$ are more resistant and recover towards baseline more quickly. This information should be helpful in trying to understand the effect of radiation therapy on anti-cancer immunity.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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