Biophysical Model of Lymphocyte Migration to Investigate Mechanisms Contributing to Radiation-Induced Lymphocyte Depletion

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BACKGROUND

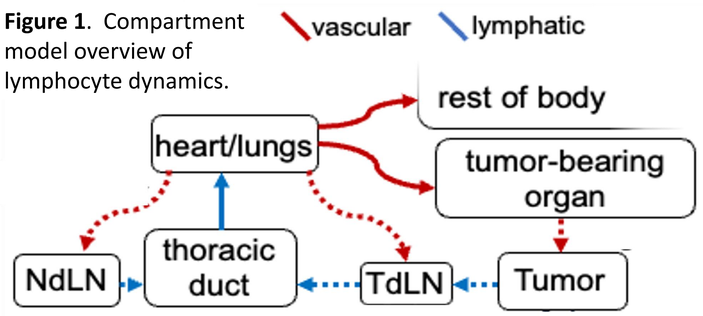
The impact of radiotherapy on circulating lymphocytes has been studied but it is unknown what exactly contributes to lymphocyte depletion. We want to understand if this observed phenomena is solely due to radiation-induced cell kill, or if other mechanisms are involved. To this end we developed a biophysical model of lymphocyte kinetics based on preclinical data.

METHODS

Experimental: Simulations were developed based on experiments in transgenic mice that express a fluorescence protein. Lymphocytes were fluorescently tagged using a 405 nm LED light source in an MC38 tumor and either left untreated (NT) or irradiated (RT) within 1h using 12Gy in a single fraction. Tagged cells were evaluated in other compartments at 24, 48, and 72h to assess dynamics.

Computational: We used a system of ordinary differential equations in python to continuously simulate lymphocyte kinetics, where transfer rates were estimated via fitting to experimental observations at 24/48/72h via least-squares. Data was taken as fractional populations of photoconverted cells and scaled by average compartment sizes. Kinetic parameters were constrained based on relative steady-state conditions of the tumor, tumor-draining lymph node (TdLN), non-draining lymph nodes (NdLN), circulation, and peripheral lymphatic organs, see **Fig 1**.

Figure 1. Compartment model overview of lymphocyte dynamics.



RESULTS

Experimental data and fits in the tumor and TdLN for CD8/CD4 subpopulations are shown in **Fig 2/3**. NT fits correspond to blue/green data, and RT fits to orange/red data. Transition rates were estimated using NT data - dashed RT lines correspond to a model using only cell kill and baseline transition rates, while solid RT lines are the results of modeling both cell kill and varying tumor outflow conditions. We determined that *both* direct cell kill and an increase in residence time in the tumor was required to explain RT data. Parameter estimates of cell kill (reported as a surviving fraction) and increases in residence time are shown in **Fig 4**.

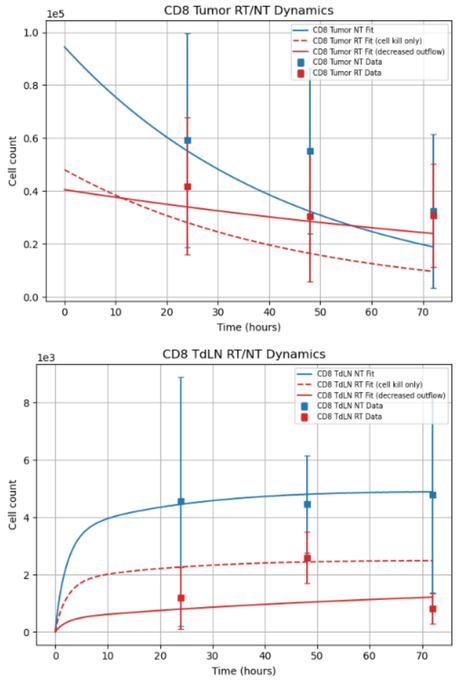
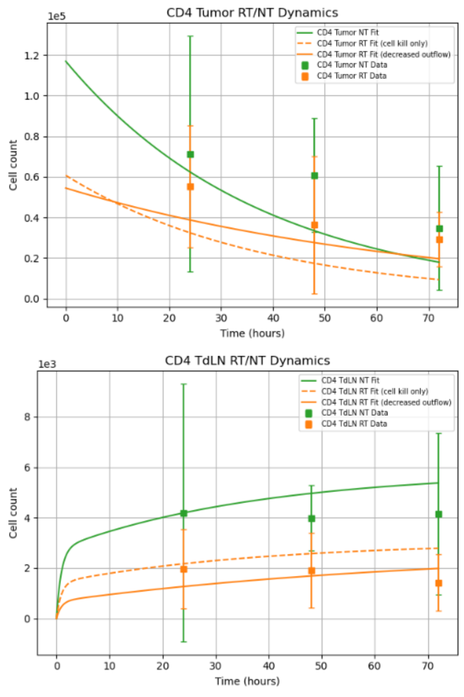


Figure 2. Fluorescently labeled CD8+ lymphocytes (above) in the Tumor and TdLN compartments. Data points are experimental fractional values scaled to compartments sizes for non-treated (NT) and treated (RT) data best model fits.

Figure 3. Fluorescently labeled CD4+ lymphocytes (below) in the Tumor and TdLN compartments. Data points are experimental fractional values scaled to compartments sizes for non-treated (NT) and treated (RT) data best model fits.



RESULTS *cont.*

Type	Model	Survival Fraction	Residence Time Increase (Tumor)	AIC
CD8	cell kill only	50.9%	0%	107
	cell kill + res	42.8%	67.4%	94.9
CD4	cell kill only	51.9%	0%	105
	cell kill + res	46.5%	45.8%	91.9

Figure 4. Review of results in CD8 and CD4 with cell kill only, and cell kill with increased residence time (cell kill+res). In treated animals, goodness-of-fit was evaluated using AIC model selection (ΔAIC>10 indicating better fit even with additional parameters).

DISCUSSION

We confirmed that our biophysical model of lymphocyte dynamics can reproduce the behavior of lymphocytes up to 72h in untreated animals, which allows us to calculate residence times and transition probabilities. After applying the baseline dynamics from untreated animals to the irradiated animals, we concluded that lymphocyte cell kill alone cannot explain the results observed in irradiated animals. Based on model fits, we can assume significant changes in egress of lymphocytes from the tumor to the TdLN.

Due to the high experimental variability of the models, further testing is needed to accurately quantify robust transition parameter estimates and evaluate lymphocyte dynamics. To address this, we plan to run simulations with different photoconversion areas and constrain parameters with additional data from non-draining lymph nodes. This model will also allow us to study the impact of different approaches to mitigate lymphocyte depletion, such as FLASH radiotherapy or dose reduction in vessels or lymph nodes.

Conclusion: We found preliminary evidence that radiation has a significant impact on lymphocyte dynamics during radiotherapy recovery periods, which could explain the large decreases in circulating lymphocytes after irradiation and the extended recovery periods we observe in patients who have been treated with radiotherapy.