Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information

Model Background

In the present study, we developed a deterministic kinetic model of CD4 T cell expansion and differentiation to provide insight into the observed synergistic effects of CRCs and TGF-β1 on T_{reg} enrichment and account for differences between observed effects on hT and mT cell differentiation. The model includes three cell populations, namely naïve CD4 T cells (T_n), effector CD4 T cells (T_e), and regulatory T cells (T_r), and the interactions between these populations are depicted in Figure 8a.

Model Governing Equations

In this model, we aimed to describe T cell developmental kinetics as simply as possible to provide a basis for treating T_{reg} manufacturing as a bioreactor and allow for direct fitting of relevant differentiation and expansion parameters based on observed phenotypic and cell expansion data. Thus, the model does not directly consider intracellular processes and broadly classifies mature non-T_{regs} as effector T cells. The differential changes in populations due to differentiation and expansion are and the resultant governing equations, as well as their analytical solutions, are summarized in Table S1.

To describe differentiation kinetics, we set up the model such that T_n cells differentiate into T_r and T_e according to the equations

$$\frac{dT_r}{dt}_{diff} = k_r T_n \#(1)$$

$$\frac{dT_e}{dt}_{diff} = k_e T_n \#(2)$$

$$\frac{dT_n}{dt}_{diff} = (-k_r - k_e)T_n \#(3)$$

where T_n, T_r, and T_e are the number of naïve, regulatory, and effector CD4 T cells respectively, and k_r and k_e are kinetic parameters that determine the rate of differentiation of T_n into T_r and T_e. To capture expansion kinetics, we use the terms

$$\frac{dT_r}{dt}expans = \frac{T_r}{t_r}\#(4)$$

$$\frac{dT_e}{dt}expans = \frac{T_e}{t_e}\#(5)$$

$$\frac{dT_n}{dt}expans = 0\#(6)$$

where t_r and t_e represent timescales for the expansion of T_r and T_e respectively. We assumed that T_n do not undergo expansion upon stimulation and serve only as a pool for T_r and T_e differentiation.

The addition of the expansion and differentiation differential terms gives the overall rate of population change for each cell type. The governing differential equations for cell growth are then

$$\frac{dT_r}{dt} = k_r T_n + \frac{T_r}{t_r}\#(7)$$

$$\frac{dT_e}{dt} = k_e T_n + \frac{T_e}{t_e}\#(8)$$

$$\frac{dT_n}{dt} = \left(-k_r - k_e\right)T_n.\#(9)$$

This system of first order linear differential equations is solvable, and results in the analytical solutions

$$T_r = -\frac{k_r T_{n0}}{k_e + k_r + \frac{1}{t_r}} e^{-\left(k_e + k_r\right)t} + \left(T_{r0} + \frac{k_r T_{n0}}{k_e + k_r + \frac{1}{t_r}}\right) e^{\frac{t}{t_r}}\#(10)$$

$$T_e = -\frac{k_e T_{n0}}{k_e + k_r + \frac{1}{t_r}} e^{-(k_e + k_r)t} + \left(T_{e0} + \frac{k_e T_{n0}}{k_e + k_r + \frac{1}{t_r}} \right) e^{\frac{t}{t_e}} \#(11)$$

$$T_n = T_{n0} e^{-(k_e + k_r)t} .\#(12)$$

To examine the role that the delay in hT cell expansion kinetics relative to mT cells plays in determining T_{reg} enrichment a modified version of the model was used in which (4) and (5) were changed to

$$\frac{dT_r}{dt}_{expans} = 0,\ t < 48;\ \ \frac{dT_r}{dt}_{expans} = \frac{T_r}{t_r},\ t \geq 48 \#(13)$$

$$\frac{dT_r}{dt}_{expans} = 0,\ t < 48; \frac{dT_e}{dt}_{expans} = \frac{T_e}{t_e},\ t \geq 48 \#(14)$$

Table S1. Model governing equations and analytical solutions.

CD4 T Cell Type	Rate of differentiation (cells/hr)	Rate of expansion (cells/hr)	Rate of population growth (cells/hr)	Population size as a function of time (cells)
Regulatory (T_r)	$k_r T_n$	$\frac{T_r}{t_r}$	$k_r T_n + \frac{T_r}{t_r}$	$-\frac{k_r T_{n0}}{k_e + k_r + \frac{1}{t_r}} e^{-(k_e + k_r)t} + \left(T_{r0} + \frac{k_r T_{n0}}{k_e + k_r + \frac{1}{t_r}} \right) e^{\frac{t}{t_r}}$
Effector (T_e)	$k_e T_n$	$\frac{T_e}{t_e}$	$k_e T_n + \frac{T_e}{t_e}$	$-\frac{k_e T_{n0}}{k_e + k_r + \frac{1}{t_r}} e^{-(k_e + k_r)t} + \left(T_{e0} + \frac{k_e T_{n0}}{k_e + k_r + \frac{1}{t_r}} \right) e^{\frac{t}{t_e}}$
Naïve (T_n)	$(-k_r - k_e)T_n$	0	$(-k_r - k_e)T_n$	$T_{n0} e^{-(k_e + k_r)t}$

Parameter Fitting

We used the analytical solutions from Table S1 along with key assumptions to directly solve for parameter values using experimental data. A summary of the parameter values in different experimental conditions may be found in Table S2.

To determine the differentiation parameter k_e, we first assumed that the factors necessary for T_e differentiation are established over the course of the first day of stimulation prior to the addition of exogenous IL-2 and any additional factors, and that T_e differentiation factors are maintained in all conditions over the course of the experiment. Thus, k_e is assumed to be constant regardless of culture conditions. Additionally, we assumed that the majority (>99.0%) of differentiation has occurred by day 4 in culture. In the absence of TGF-β1, we assume that there is no T_r differentiation and that k_r is negligible. Combined, this results in the simplification of (12) to

$$T_n = T_{n0} e^{-(k_e)t} \#(15)$$

in control conditions which allows for the direct solving of k_e. The resulting k_e value may be used in conjunction with (12) to find k_r using data from TGF-β1 only data.

To determine the expansion timescale parameters t_r and t_e we assume that (i) the rate of expansion is dependent directly on the number of cells of the population and (ii) is zero on the first day prior to factor addition and constant for each subsequent day. Due to lack of differentiation of T_r in the control condition, (10) simplifies to

$$T_r = T_{r0} e^{\frac{t}{t_r}} \#(16)$$

and we can directly solve for t_r using control condition experimental data. Additionally, we can solve for the value of t_e in the presence and absence of CRCs using (11) in combination with data from control and CRC only experiments. An Excel sheet with example calculations may be found at https://github.com/Shah-Lab-UCSD/Treg_Enrichement_Model_In_Vitro.

Table S2. Experimentally determined model parameter values

Parameter	Control	1 μM CRCs	TGF-β1	1 μM CRCs +

				TGF-β1
k_e (1/hr)	0.06396	0.06396	0.06396	0.06396
k_r (1/hr)	0	0	0.05638	0.05638
t_e (hr)	49.59	61.23	49.59	61.23
t_r (hr)	44.75	44.75	44.75	44.75

Comparison to Similar Models

This model of expansion is comparable to a previously proposed model proposed in Mayer et al. in which T cell expansion is governed according to the equation

$$\frac{dT}{dt}_{expans} = \alpha \frac{TC}{K + T + C} \#(17)$$

in which α is the maximal rate of expansion, and K, T, and C are values determined by the antigen affinity, T cell number, and concentration of cognate antigen[1]. Relative to this model, the present model value of C, determined by the concentration of Dynabeads, is assumed to be much larger than K or T, so (17) simplifies to

$$\frac{dT}{dt}_{expans} = \alpha T \#(18)$$

where the rate of expansion is determined to only be dependent on the T cell number and the maximal rate of expansion α, which is analogous to the reciprocal of t_r or t_e in the current model depending on the cell type. It should be noted that the characteristic doubling times reported here are slower than reported literature values (36 hr ± 7.2 hr) for T cells undergoing peak expansion in comparable studies. This may be due to the use of Dynabeads over a TCR specific cognate antigen, but also likely arises as a result of the assumption of a constant rate of expansion in the present model. However, from a manufacturing perspective, it is more convenient to deal with a time averaged expansion rate than consider instantaneous expansion rate, and as all experiments

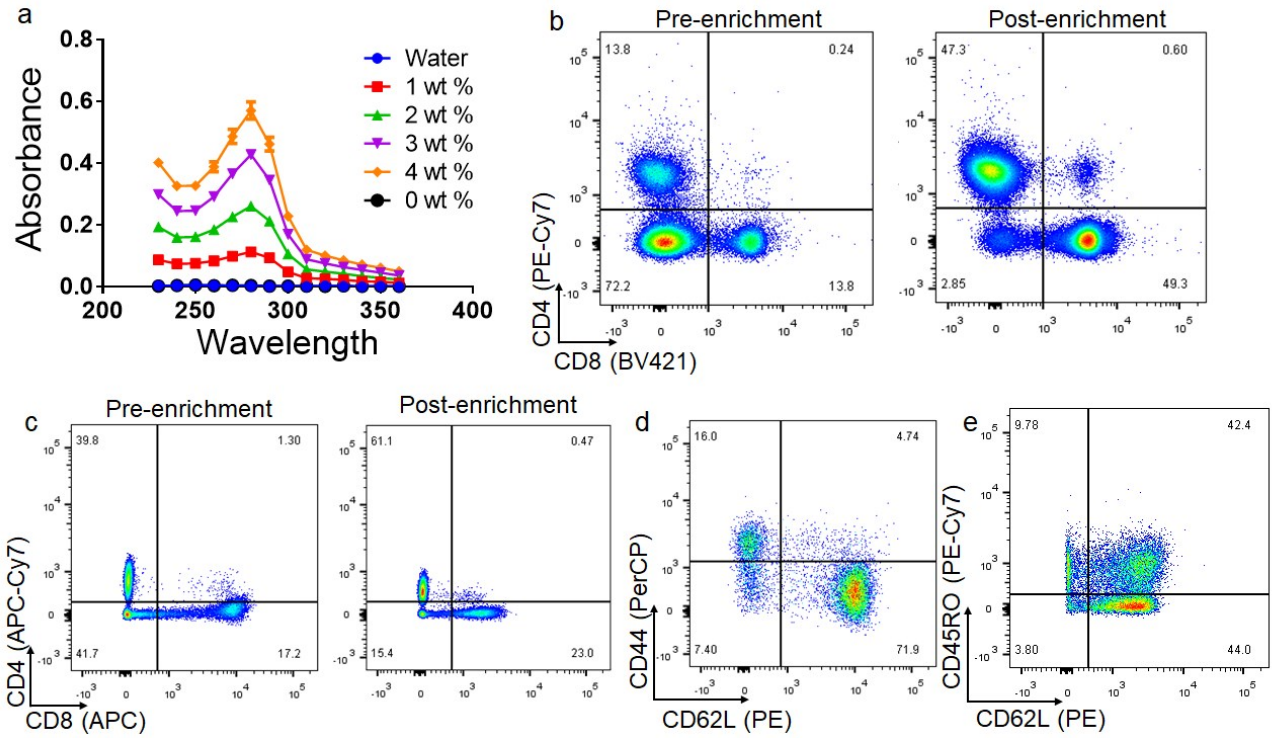
were conducted over a 7-day period with the same amount of stimulation, the value still allows for comparison between groups.

Reference

1. A. Mayer, Y. Zhang, A. S. Perelson and N. S. Wingreen, *Proceedings of the National Academy of Sciences of the United States of America*, 2019, **116**, 5914-5919.

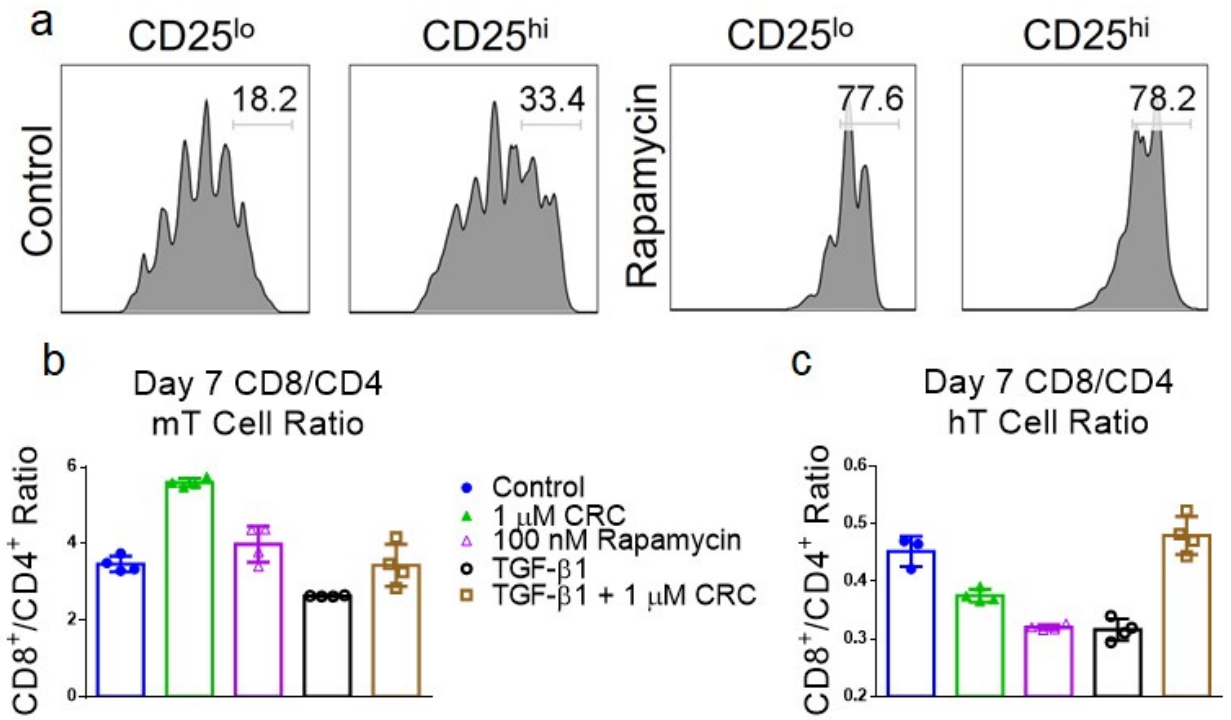
Supplementary Figures

Figure S1



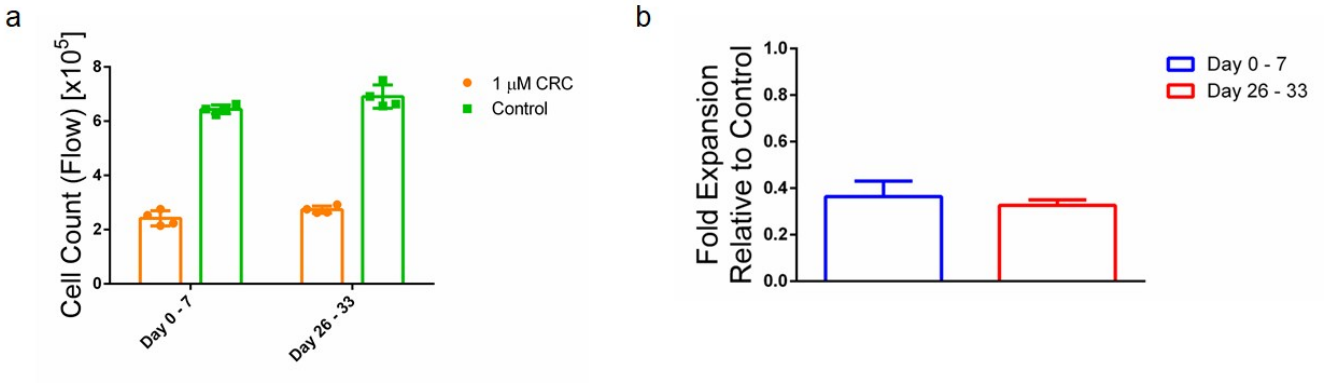
Extended characterization of CRCs and freshly isolated T cells. (a) Representative absorbance curves from spectrophotometric analysis of CRCs made with increasing concentrations of βCD. (b) Representative flow cytometry plots of murine splenocytes pre- and post-enrichment for T cells. (c) Representative flow cytometry plots of human PBMCs pre- and post-enrichment. (d) Representative flow cytometry plot for $CD4^+CD62L^+CD44^-$ naïve mT cells. (e) Representative flow cytometry for $CD4^+CD62L^+CD45RO^-$ naïve hT cells. In a, data represents mean ± s.d. (n=3) of technical replicates.

Figure S2



Extended characterization of T cell expansion. (a) Representative CFSE plots gated on $CD4^+$ $CD25^{lo}$ and $CD4^+CD25^{hi}$ T cells. (b) $CD8^+/CD4^+$ ratio in murine T cells after 7 days of expansion. (c) $CD8^+/CD4^+$ ratio in hT cells after 7 days of expansion.

Figure S3



Extended characterization of CRC bioactivity. Quantification of 1μM CRC-mediated suppression of hT cell expansion relative to proliferation of Dynabead-only controls of experiments starting at 0 and 26 days after initial preparation of CRCs. Data is represented as (a) total flow cytometry counts and (b) fold-expansion of CRC treated groups relative to controls. CRCs were stored at 4^{o}C and warmed to room temperature and vortexed before addition to culture media. Data represent the mean ± s.d. (n = 4).