RESEARCH ARTICLE

Direct effects of interleukin-7 on the function of human T cells *in vitro*

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ABSTRACT. CD3+ T lymphocytes were isolated by positive magnetic separation from the peripheral blood of healthy donors. In the absence of any additional activating stimuli, interleukin-7 (IL-7) was shown to augment the levels of T cells expressing CD25 activation marker both in CD4-positive and in CD4-negative effector memory ($CD45RA^-CD197^-$) T cell subsets, as well as in terminally differentiated ($CD45RA^+CD197^-$) T cells, without significantly affecting the activation status of naive ($CD45RA^+CD197^+$) and central memory ($CD45RA^-CD197^+$) T cells. In addition, IL-7 noticeably enhanced the production of IL-2, interferon-γ (IFN-γ), and IL-10, but not IL-4, in T cells. The direct effects of IL-7 on T cell activation induced *in vitro* by MACSiBead^{TM} particles coated with CD2, CD3, and CD28 antibodies (Abs) were also investigated. Upon cell activation, IL-7 significantly augmented the levels of CD25+ T cells in naive (CD45RA+CD197+), central memory ($CD45RA^-CD197^+$), and effector memory ($CD45RA^-CD197^-$) T-cell compartments. In addition, IL-7 facilitated activation of $CD4^-$ (but not $CD4^+$) terminally differentiated effector ($CD45RA^+CD197^-$) T cells. Finally, IL-7 was found to upregulate the production of IL-2, IFN-γ, IL-4, and IL-10 by activated T cells. In conclusion, we speculate that IL-7 is capable of enhancing functional T cell activity without causing significant functional inbalance between various T cell subsets.

Key words: interleukin-7, T cell, homeostatic activity, T-cell activation, CD25, cytokine production

Interleukin-7 (IL-7) is a lymphopoietic cytokine with mol. weight 25 kDa, which mainly affects T- and B cells, as well as natural killer (NK) cells [1-3]. IL-7 is produced manly by nonhematopoietic cells (fibroblastic stromal cells in the bone marrow and thymus and keratinocytes in the skin), as well as by epithelial cells (in the thymus, prostatic epithelium, and the intestine) and smooth muscle cells. Dendritic cells and macrophages are known to produce IL-7 in much smaller quantities [4].

IL-7 acts as an indispensable survival factor for naive T cells; it also supports the homeostatic proliferation of memory T cells *via* downregulating their apoptotic activity and upregulating their growth activity [5]. Membrane expression of IL-7 receptor α-chain (CD127) determines the sensitivity of T cells to IL-7, while IL-7 receptor γ-chain (CD132) mediates its functional activity; the IL-7 receptor γ-chain is shared by a variety of cytokines with growth factor activity (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21) [4, 6-8].

Thus far, the expression of IL-7 receptor has been detected on nearly all T-cell subpopulations including regulatory T cells [9, 10]. Current knowledge maintains that both homeostatic and antigen-induced lymphocyte proliferation occurs mainly in lymphoid organs. Therefore, theoretically, T cells expressing CCR7 (CD197) chemokine receptor, which controls migration and homing of T cells to lymphoid organs [11], should be most sensitive to IL-7. In this study, we aimed:

– to assess direct effects of IL-7 on activation status of CCR7-positive and CCR7-negative T cells,

– to characterize direct effects of IL-7 on T-cell production of pro-inflammatory and anti-inflammatory cytokines *in vitro*.

Abbreviations

Ab	antibody
IFN	interferon
IL	interleukin
PBMC	peripheral blood mononuclear cell
Th	helper T cell
Treg	regulatory T cell

MATERIALS AND METHODS

This study was approved by the Scientific Council and Ethics Committee of Immanuel Kant Baltic Federal University (protocol N° 7, 10.03.2015). Informed consent was obtained from every subject enrolled in this study.

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Isolation of T cells

This study recruited 14 healthy male and female donors aged between 21 and 40 years. Peripheral blood mononuclear cells (PBMC) were isolated using Ficoll-Paque (Ficoll-Paque™ PREMIUM, 1.077 ± 0.001 g/mL, GE Healthcare, USA) gradient centrifugation, and cells were counted using a Z2 Cell and Particle counter (Beckman Coulter Inc., Fullerton, CA). T cells were positively selected using human CD3 MACS® MicroBeads and MS Columns (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) according to the manufacturer's instructions.

T-cell cultures

Isolated T cells were cultured at 1.0-1.5 × 10^6/mL in the presence or absence of MACSiBead particles conjugated with antibodies (Abs) against human CD2, CD3, and CD28 (T Cell Activation/Expansion Kit, human, MACS Miltenyi Biotec) in a serum-free medium developed for the cultivation and expansion of T cells (TexMACS™, Miltenyi Biotec) in 24-well plates in a humidified CO2 incubator at 37°C for 48 h. In parallel, serial 10-fold dilutions (range 0.01-10 ng/mL) of human IL-7 (research grade, Miltenyi Biotec) were added to some T-cell cultures.

T-cell identification

After being cultured, T-cell subsets were identified by flow cytometry using cocktails of fluorochrome-labeled monoclonal Abs prepared *ex tempore*: fluorescein isothiocyanate (FITC)-labeled anti-CD4 Ab (eBioscience, San Diego, CA), phycoerythrin (PE)-labeled anti-CD197 (BioLegend, San Diego, CA), allophycocyanin (APC)-labeled anti-CD45RA (BD Pharmingen, San Jose, CA), peridinin chlorophyll (PerCP)-labeled CD25 (BioLegend). FcR Blocking Reagent (Miltenyi Biotec) was used to block nonspecific binding of Abs. Fluorescence Minus One Control (FMO) was used to optimize gating strategy and account for the nonspecific Ab binding in the PerCP (CD25) channel (cocktail of antibodies prepared *ex tempore:* CD4-FITC, CD197-PE, CD45RA-APC). The level of nonspecific background signal caused by primary Abs was measured using an isotype control (ISO 25 PerCP Mouse IgG1, κ, BioLegend). Flow cytometry was performed on a BD Accuri™ C6 Flow Cytometer.

Cytokine measurement

Cell culture supernatants were collected and concentrations of IL-2, IL-4, IL-10, and IFN-γ were measured using commercially available ELISA kits (Vector-Best, Novosibirsk, Russian Federation) according to the manufacturer's instructions using an Automated EIA and Chemistry Analyzer (ChemWell 2910, Awareness Technology, Inc., Palm City, FL).

Statistics

Statistical analysis was performed using IBM SPSS Statistics 21 (SPSS, Statistical Package for the Social Sciences IBM Corporation, NY, USA). For each cohort, median (Me), first and third quartiles (25; 75) were calculated. A nonparametric Wilcoxon test was used to compare related samples. Linear regression analysis was used for estimating the relationship between the independent and dependent variables. $P < 0.05$ was considered statistically significant.

RESULTS

Purity and viability of isolated T cells following positive selection with human CD3 MACS® MicroBeads was assessed using PE-labeled anti-CD3 Ab (eBioscience) in the presence of a membrane impermeable dye propidium iodide (PI) (eBioscience) applied for staining dead cells. *Figure 1* shows that purity of T cells in the positively selected T-cell population was 99.0 ± 0.9%, with cell viability not less than 95%.

Isolated T cells were cultured with or without MACSiBead particles bearing Abs against human CD2, CD3, and CD28, which have been formulated to result in an optimal activation of T cells. Following 48 h cultivation, T-cell viability was not lower than 90%. As described in detail previously [12], the gating strategy applied in this study allowed for the efficient identification of $CD4^+$ and $CD4^-$ T cells, as well as the subdivision of T cells into naive T cells ($CD3^+CD45RA^+CD197^+$), central memory T cells ($CD3^+CD45RA^-CD197^+$), effector memory T cells ($CD3^+CD45RA^-CD197^-$), as well as terminally differentiated effector T cells ($CD3^+CD45RA^+CD197^-$). The expression of the IL-2 ($CD25^+$) receptor α-chain activation marker [1] was determined in each of the T-cell subsets studied.

Table 1 shows that culturing T cells with IL-7 (range 1.0-10.0 ng/mL) in the absence of any additional activating stimuli resulted in a statistically significant increase in the levels of $CD25^+$ T cells both in CD4-positive and in CD4-negative T-cell compartments. The effect of IL-7 was dose-dependent ($R^2 = 0.580$, $P < 0.05$ and $R^2 = 0.647$, $P < 0.05$ for $CD4^+$ and $CD4^-$ T cells, respectively). The data denotes that the observed increase in $CD25^+$ T cells in $CD4^+$ and $CD4^-$ T cell subsets was almost entirely accounted for by effector memory T cells ($CD45RA^-CD197^-$). It was only at the highest concentration of IL-7 tested (10 ng/mL) that we observed also a marginal increase in $CD25^+$ T cells in the terminally differentiated effector T-cell ($CD3^+CD45RA^+CD197^-$) compartment.

IFN-γ production is known to characterize T-helper 1 (Th1) cell activity, whereas IL-4 is produced by Th2 T cells and IL-10 predominantly by regulatory T (T reg) cells [1]. Data shown in *table 2* suggests that IL-7 noticeably facilitated the production of IL-2, IFN-γ, and IL-10 by T cells without affecting the levels of IL-4. The effects of IL-7 on cytokine production by T cells in this model were most pronounced at the highest IL-7 concentration tested (10 ng/mL).

The expression of CD25 marker was also determined in T cells cultured with the activating particles. *Table 3* shows that IL-7 promoted activation of all T-cell subsets identified with a notable exception of terminally differentiated effector T cells ($CD4^+CD45RA^+CD197^-$). Effector memory T cells ($CD4^-CD45RA^-CD197^-$) and terminally differentiated effector T cells ($CD4^-CD45RA^+CD197^-$) were found to be most sensitive to the direct action of IL-7 in *in vitro* experiments.

Data presented in *table 4* shows that IL-7 facilitated the production of IL-2/IFN-γ and IL-4/IL-10 cytokines by

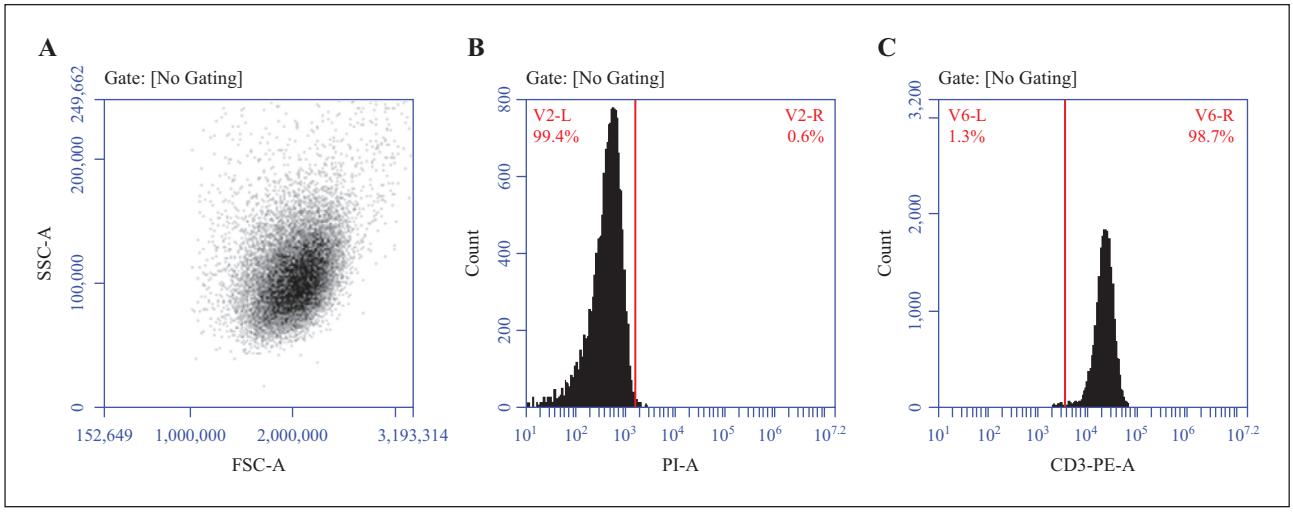

Figure 1
Evaluation of cell purity and viability in purified CD3⁺ T-cell population. **A.** A scatter plot of forward scatter (FSC) > *vs* side scatter (SSC) was used to assess size-dependent and complexity-dependent cell distribution. **B.** A single-parameter histogram of PI staining (dead cells) positive staining shows dead cells, negatively stained cells represent live cells. **C.** A single-parameter histogram showing purity of isolated CD3⁺ T-cell population.

Table 1
Percentage of CD25 ⁺ T cells in T-cell subsets cultured without MACSiBead particles.

T cells	IL-7 (ng/mL)				
	0	**0.01**	**0.10**	**1.00**	**10.00**
$CD3^+$	0.1 (0-0.2)	0.1 (0.1-0.2)	0.2 (0.1-0.3)	0.5* (0.3-0.8)	3.1* (1.2-4.4)
$CD4^+$	0.1 (0-0.1)	0.1 (0-0.3)	0.1 (0-0.3)	0.4* (0.2-0.7)	2.4* (0.7-4.2)
$CD45RA^+CD197^+$	0	0	0	0 (0-0.1)	0.1 (0-1.3)
$CD45RA^-CD197^+$	0	0	0 (0-0.1)	0.1 (0-0.4)	0.8 (0-3.4)
$CD45RA^-CD197^-$	0.1 (0-0.2)	0.2 (0-0.6)	0.2 (0-0.7)	1.1* (0.2-2.4)	5.1* (1.6-12.1)
$CD45RA^+CD197^-$	0 (0-1.1)	0 (0-0.1)	0 (0-0.1)	0 (0-0.9)	1.0 (0-2.6)
$CD4^-$	0.2 (0 -0.3)	0.2 (0.1-0.2)	0.2 (0-0.5)	0.6* (0.3-1.6)	3.6* (1.8-5.3)
$CD45RA^+CD197^+$	0 (0-0.1)	0	0	0 (0-0.1)	0.3 (0-0.5)
$CD45RA^-CD197^+$	0 (0-0.3)	0 (0-0.1)	0 (0-0.2)	0.6 (0-3.5)	3.5 (0-6.8)
$CD45RA^-CD197^-$	0.3 (0-0.6)	0.4 (0.2-0.6)	0.6 (0-1.3)	2.1* (0.5-4.3)	9.6* (6.1-14.6)
$CD45RA^+CD197^-$	0.4 (0-0.9)	0.2 (0-0.4)	0.1 (0-0.5)	0.3 (0-1.4)	2.3* (0.7-5.0)

$P < 0.05$ - as compared to T cells cultured in the absence of IL-7.

activated T cells. Furthermore, IFN-γ production exhibited the highest sensitivity toward IL-7 stimulatory signals, such that even the lowest IL-7 concentrations used (0.01 ng/ml) resulted in a statistically significant augmentation of IFN-γ production in T cells.

DISCUSSION

Both IL-7 and IL-15 play a pivotal role in maintaining homeostatic proliferation of lymphoid cells. Interactions between IL-7 and its receptor have been shown to stimulate signal transducer and activator of transcription 5 (STAT5) phosphorylation and activation of PI3K/P-Akt-dependent signal transduction pathway [6-8]. There is evidence to suggest that IL-7 could induce CD25 expression on T cells, thus sensitizing them to IL-2 stimulation. This IL-7-dependent sensitization contributes to self-maintenance of T cells and increases their alertness to antigenic stimulation [13, 14]. Since lymphoid organs constitute the major site for T-cell proliferation and differentiation, we hypothesized that naive T cells and central memory T cells possess

Table 2
Concentration of cytokines (pg/mL) in T-cell cultures without MACSiBead particles.

Cytokine	Concentration of IL-7 (ng/mL) in T-cell cultures				
	0	**0.01**	**0.10**	**1.00**	**10.00**
IL-2	< 5	< 5	< 5	32* (7-139)	54* (33-88)
IFN-ã	< 10	< 10	< 10	36 (10-636)	911* (10-2481)
IL-4	< 1	< 1	< 1	< 1	< 1
IL-10	< 3	< 3	< 3	< 3	46* (3-118)

$P < 0.05$ - as compared to T cells cultured in the absence of IL-7.

a relatively high sensitivity profile to IL-7; those T cells are known to express CCR7 (C197) chemokine receptor, which controls T-cell migration and homing to the lymphoid organs, where T cells undergo antigen-driven activation and differentiation [1]. However, IL-7 was found in this study to augment the levels of $CD25^+$ T cells within CCR7-negative effector memory and terminally differentiated T-cell compartments, which was in disagreement with our hypothesis. Indeed, based on our results, it is tempting to speculate that it is the direct effect of IL-7 on T cells that can guarantee their survival in the periphery in the absence of membrane-associated and/or cytokine-modulated costimulatory signals, as well as create conditions necessary for implementing their effector functions upon antigen encounter. It is worth mentioning that our experimental model is devoid of accessory cells, thus adequately modeling the conditions in periphery characterized by a relative deficit in the costimulatory signals that could be potentially involved in T-cell activation. Indeed, previous reports described a synergistic signaling effect of IL-7 and OX40 (CD134, tumor necrosis factor receptor superfamily, member 4, TNFRSF4) on T cells, and the expression of OX40L on accessory cells [15]. In this context, we envisage that activating effects of IL-7 on naive T cells and central memory T cells described previously could have reflected the presence of accessory cells (and hence optimal costimulation signals) in the experimental models applied [14, 16].

Since the T cells were purified using anti-CD3 Ab conjugated with MACSiBeads, we cannot fully extrapolate the data from our experimental model to homeostatic T-cell proliferation. Indeed, anti-CD3 binding alone could lead to partial (suboptimal, marginal) activation of the T cells, and, thereby, could induce activation-induced cell death (AICD) within a short period of time. In our experiments, IL-7 could help these "marginally stimulated" T cells to escape from AICD through stimulating production of growth factors and cell expression of viability molecules, such as IL-2 receptor and B-cell lymphoma 2 (BCl2) regulator protein. Indeed, our data indicated that IL-7 was able to induce IL-2 production and CD25 expression on T cells in the absence of additional stimulation. Hence, we hypothesize that IL-7 may create prerequisites necessary for survival and self-maintenance of effector T cells in the periphery when there is a deficit in immune-mediated cytokine and membrane costimulation.

Cultivation of T cells in the presence of IL-7 induced IFN-γ (but not IL-4) production, which can indicate a

Table 3
Percentage of $CD25^+$ cells in T-cell subsets cultured with MACSiBead particles.

T-cell subpopulation	Without activation	Activation + IL-7 (ng/mL)				
		0	**0.01**	**0.10**	**1.00**	**10.00**
$CD3^+$	0.1 (0-0.2)	9.7* (4.8-13.4)	13.2** (8.7-15.9)	13.4** (5.7-18.9)	13.2** (8.3-20.4)	13.9** (8.8-19.7)
$CD4^+$	0.1 (0-0.1)	6.9* (3.9-10.1)	9.6** (4.3-12.6)	8.3** (4.3-13.9)	10.2** (7.4-13.0)	10.1** (5.7-14.3)
$CD45RA^+/CD197^+$	0	2.6* (1.5-4.6)	3.8** (2.2-4.9)	3.6 (2.4-4.8)	5.2** (2.9-7.7)	4.6 (2.1-7.0)
$CD45RA^-/CD197^+$	0	8.4* (5.8-12.4)	9.6 (6.4-13.0)	9.3 (5.7-14.9)	12.0 (5.8-16.4)	13.0** (6.0-16.9)
$CD45RA^-/CD197^-$	0.1 (0-0.2)	26.0* (5.6-36.5)	37.1 (5.3-40.1)	27.5 (6.6-49.8)	32.8** (10.8-47.1)	36.2** (9.9-49.5)
$CD45RA^+/CD197^-$	0 (0-1.1)	17.3* (3.2-33.7)	21.5 (1.8-29.0)	18.5 (4.6-31.7)	16.3 (5.4-36.0)	26.5 (6.6-35.7)
$CD4^-$	0.2 (0-0.3)	13.1* (5.0-24.3)	22.1** (9.8-27.6)	23.8** (5.1-32.6)	24.5** (7.5-34.4)	23.6** (8.2-35.5)
$CD45RA^+/CD197^+$	0 (0-0.1)	2.6* (1.5-6.9)	5.3 (1.8-7.3)	5.8** (2.1-10.2)	5.3** (3.5-10.9)	7.2** (3.5-12.4)
$CD45RA^-/CD197^+$	0 (0-0.3)	18.6* (13.2-30.4)	23.7 (19.0-27.9)	32.2** (19.8-2.1)	36.9** (24.2-42.6)	35.0** (27.7-8.7)
$CD45RA^-/CD197^-$	0.3 (0-0.6)	23.2* (9.8-35.1)	34.4** (18.8-40.6)	36.5** (12.6-48.2)	39.8** (18.9-48.7)	38.5** (21.5-0.5)
$CD45RA^+/CD197^-$	0.4 (0-0.8)	16.0* (9.6-18.2)	22.7** (13.6-27.4)	23.6** (9.6-29.8)	23.3** (10.9-28.7)	26.2** (14.2-8.5)

* $P < 0.05$, as compared with T cells cultured in the absence of MACSiBead particles and IL-7.
** $P < 0.05$, as compared with T cells cultured in the presence of MACSiBead particles and in the absence of IL-7.

Table 4
Concentration of cytokines (pg/mL) in T-cell cultures with MACSiBead particles.

Cytokine	Without activation	Activation + IL-7 (ng/mL)				
		0	**0.01**	**0.10**	**1.00**	**10.00**
IL-2	<5	1071* (132–2664)	1542 (360-2398)	1048** (292-3150)	1150** (570-4106)	1872 (472–5654)
IFN-ã	<10	2090* (1379-3728)	2899** (2139-6089)	6250** (2587-9992)	5642** (2392-9124)	6332** (3620-9842)
IL-4	<1	31* (7-47)	37 (11-53)	60** (35-70)	85** (62-96)	90** (63-120)
IL-10	<3	330* (131-680)	429 (149-668)	578** (164-926)	792** (277-1266)	779** (386-1345)

* $P < 0.05$, as compared with T cells cultured without MACSiBead particles and IL-7.
** $P < 0.05$, as compared with T cells cultured with MACSiBead particles and in the absence of IL-7.

pro-inflammatory bias of IL-7 activity, thus contributing to shaping a state of alertness of the immune system and its readiness to form Th1-mediated cellular immune responses to antigenic stimulation.

The former paradigm maintains that regulatory T cells normally fail to express CD127 and therefore are generally resistant to IL-7 stimulation. However, it is now well established that T cells do express IL-7 receptors in quantities sufficient for signal transduction and downstream modification of cell functions. Moreover, IL-7 has been shown to play a pivotal role in the homeostatic maintenance of regulatory T cells [10], which are considered the major T-cell subpopulation capable of IL-10 production [1]. Hence, our data further develops the concept of the IL-7/T-cell interrelationship in showing that IL-7 is able to upregulate directly IL-10 production in T cells. We speculate that IL-7 could therefore potentially restrain the excessive growth of T cells and maintain their clonal balance in the body.

This study showed that IL-7 was capable of promoting activation of all T-cell subsets studied with a notable exception of terminally differentiated effector $CD4^+$ T cells. On the other hand, both terminally differentiated $CD4^-$ T cells and effector memory $CD4^-$ T cells were found to be most sensitive to low IL-7 concentrations. According to our study, IL-7 upregulated IL-2, IFN-γ, IL-4, and IL-10 production by activated T cells. In this respect, it is important to emphasize that IL-7 was also facilitating an increase in IL-2, IFN-γ, and IL-10 (but not IL-4) production by T cells that were cultured in the absence of any additional activating stimuli. However, the effects of IL-7 on the upregulation of cytokine production in such T cells were less pronounced, as compared with activated T cells. Interestingly, it was the production of IFN-γ by T cells that exhibited the highest sensitivity to IL-7 in our *in vitro* experiments. We envisage that a significant contribution toward IFN-γ production in our experimental model originated from CD4-negative T cells that are capable of mounting responses in the presence of low concentrations of IL-7.

It is worth mentioning that our experimental model excluded accessory cells, thus allowing for modeling a relative deficiency of cytokine milieu potentially involved in T-cell activation, thus matching the particular conditions existing in the tumor microenvironment.

Altogether, the experimental data presented herein suggests the involvement of IL-7 in the direct upregulation of growth and functional activity of T cells. In this scenario, IL-7 is likely to facilitate a balanced activation of various T-cell subsets and downstream formation of adequate immune responses.

CONCLUSION

IL-7 is able to provide direct positive regulation of functional T-cell activity without causing significant functional inbalance between various T-cell compartments.

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