Biochemistry and Biophysics Reports 7 (2016) 164–172

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

T cell up-regulation of CD127 is associated with reductions in the homeostatic set point of the peripheral T cell pool during malnourishment

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ARTICLE INFO

Article history:
Received 11 March 2016
Received in revised form
2 June 2016
Accepted 6 June 2016
Available online 8 June 2016

Keywords:
Malnutrition
Homeostasis
T cells
Lymph nodes
Immunodeficiencies

ABSTRACT

The following study was undertaken to better understand the mechanisms that relate the homeostatic set point of the peripheral T cell population to energy availability in mice. We report that the total number of peripheral naïve and memory CD4+ and CD8+T cells notably declined after one week of malnourishment, a time period too short to be entirely due to malnutrition-induced thymic involution. Peripheral malnourished T cells expressed higher levels of the IL-7 receptor component, CD127, and were less sensitive to death-by-neglect as compared to control T cells. Overall levels of IL-7 were similar in malnourished and control mice. Adoptive transfer studies revealed that CD127 expression did not correlate with increased survival *in vivo* and that all naïve CD8+T cells upregulated CD127, regardless of initial expression levels. Corticosterone levels were elevated in malnourished mice and this correlated in time with peripheral T cell up-regulation of CD127 and the diminishment of the peripheral T cell pool. Overall, these data suggest a model in which CD127 levels are up-regulated quickly during malnourishment, thereby increasing the scavenge rate of IL-7, and providing a mechanism to quickly adjust the total number of T cells during malnutrition.

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1. Introduction[1]

Malnutrition is the leading cause of immunodeficiency worldwide [1]. Indeed, malnutrition and infection are intricately linked as malnourished children not only experience a heightened risk of developing infections such as diarrhea and pneumonia, but also experience greater morbidity from infection [2]. Furthermore, the nutrition of infected children is negatively impacted by a multitude of factors, including diarrhea, malabsorption, loss of appetite, diversion of nutrients for the immune response, and urinary nitrogen loss [1]. Consequently, infections further exacerbate malnutrition and immunodeficiency in the malnourished. Quite unfortunately, reports suggest that the interaction between malnutrition and infection contributes to 53% of all child deaths in developing countries [3]. Additionally, malnutrition-related immunodeficiency likely reduces the efficacy of certain vaccines, such as those for rotavirus, polio, and cholera [4–6].

Malnutrition results in notable changes to the size, composition, and overall function of the T cell compartment [7]. In order for the immune system to respond to any pathogen that may be encountered, the maintenance of a diverse pool of antigen-inexperienced naïve T cells is critical [8]. The size of the naïve T cell pool is determined by the rate of egress from the thymus as well as by peripheral T cell survival and proliferation. During malnutrition, extreme thymocyte depletion occurs due to increases in thymocyte death and decreases in thymocyte proliferation [9]. Interestingly, studies using protein-malnourished mice have demonstrated atrophy of the thymus may be directly caused by increases in glucocorticoid levels and/or decreases in leptin, the satiety hormone [10–12]. The number of peripheral T cells is also reduced in malnourished individuals, and this reduction has been attributed to reduced thymic input [9]. The possibility of additional, peripheral mechanisms that contribute to resetting the homeostatic set point of the naïve CD8+T cell pool during malnourishment has not been addressed.

In addition to diminishment in the size of the T cell population, the T cells that remain in malnourished individuals are functionally impaired [9]. For example, T cell dependent immune reactions such as delayed hypersensitivity responses, are markedly suppressed and are commonly absent in malnourished children [13]. However, existing B cell numbers and function seem to be

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[1] MAL – malnourished; AL – *ad libitum*; LN – lymph node; MHC I – major histocompatibility complex I; IFN-γ – interferon gamma

http://dx.doi.org/10.1016/j.bbrep.2016.06.006
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surprisingly maintained with only new B cell responses to T cell dependent antigens experiencing impairment [10]. This suggests that malnourishment does not affect B cell function directly and only influences antibody responses that require T cell help. Thus, malnourishment impairs the T cell arm of the adaptive immune response by diminishing their number and function.

In the periphery, individual T cells are maintained in response to extrinsic cues rather than an intrinsic survival clock. Specifically, the total number of naïve T cells is continuously adjusted through homeostatic signaling processes that involve self-peptide MHC-complexes and interleukin-7. Indeed, naïve T cells do not survive well in IL-7 or MHC Class I deficient mice [14–17]. Interestingly, it appears that the dose of IL-7 received by the naïve T cell influences how long that cell can persist in the absence of further survival factors, at least in *ex vivo* studies [18].

A key survival factor for T cells, IL-7 is secreted by fibroblastic stromal cells in the bone marrow and lymph nodes as well as by epithelial cells in the thymus and intestine [19]. The available amount of IL-7 in the central lymphoid organs was thought to be primarily regulated by the rate that it is scavenged by T cells [20]. However, recent studies using IL-7 reporter mice suggest that IL-7 expression is increased by lymph node stromal cells during lymphopenia [21]. In certain contexts, some cytokines can also affect production of IL-7 *in vivo* [22–24]. Thus, although the scavenge rate may influence IL-7 availability, extrinsic stimuli such as cytokines and lymphopenic conditions influence the rate of IL-7 production, at least by stromal cells.

T cells detect IL-7 *via* the IL-7 receptor, which is a heterodimer comprised of CD127 (also known as IL-7Rα) and the common gamma chain (γ_c) [25]. T cell expression of CD127 is thought to be highly regulated; CD127 is up-regulated in the absence of IL-7 and conversely down-regulated after contact with it [25]. In addition, glucocorticoids, type I interferons, and TNF-α can up-regulate CD127 expression; while several cytokines (many of which signal through receptors partially comprised of γ_c) down-regulate CD127 in T cells [20].

As aforementioned, maintaining a large diversity of adaptive immune cells is necessary for survival given the unknown nature of pathogens that might be encountered. Considering the energetic challenges that this adaptation poses, lymphocytes are maintained as naïve, quiescent cells in low numbers until particular cells are called upon to defend against a cognate antigen. However, it is less clear how T cell homeostasis is linked to energy availability during drastic energy shortages, as occurs in malnourishment. The following study was undertaken to better understand the impact of short-term malnutrition on T cell homeostasis, with particular attention focused on the CD127/IL-7 signaling dynamic of peripheral T cells.

2. Materials and methods

2.1. Mice

All mice were housed in the mouse facility at Randolph-Macon College in accordance with approved Institutional Animal Care and Use Committee guidelines and protocols and with the NIH guide for the care and use of laboratory animals. For each individual experiment, the mice were placed on malnourishment as follows. Male and female C57BLJ/6 mice ranging in age from 8 to 15 weeks old were singly housed. The unrestricted intake of chow was monitored for each mouse two weeks prior to the initiation of malnourishment. Mice were randomly assigned to each treatment group. Malnourished (MAL) mice received 35% less Teklad Global 18% Protein Rodent Diet (Harlan Laboratories) by weight while *ad libitum* (AL) control mice had unrestricted access to the same chow. The dieting period lasted one week for all studies with the exception of the "death-by-neglect" study in which the dieting period lasted 6 weeks. Although some individual malnourished mice lost up to 28% of their body weight, on average, malnourished mice lost 10%, while control mice gained 3% of their original body weight over the course of one week. In all experiments, mice were euthanized *via* CO_2 overdose followed by cervical dislocation.

2.2. Flow cytometry

After 1 week of the malnourishment or control diet, cells were isolated from the spleens of male and female C57BL/6J mice aged 10–13 weeks after a malnourished or control diet for one week. Red blood cells were removed from splenocyte preparations by incubation with ACK lysing buffer (0.15 M NH_4Cl; 10 mM $KHCO_3$; 0.1 mM EDTA). The ammonium chloride in the buffer lyses red cells with minimal effect on lymphocytes. After osmotic lysis, one million cells were incubated with Fc-blocking reagent (Purified Anti-Mouse CD16/CD32 (2.4G2)) (Tonbo, California, USA). Surface protein markers used to distinguish particular immune cell subsets are as follows: T cells (CD3+ (145–2C11, eBioscience, California, USA)), B cells (CD19+ (eBio1D3, eBioscience, California, USA)), granulocytes (CD11b+ (M1/70, eBioscience, California, USA) Gr1+ (RB6–8C5, Invitrogen, USA)), and monocytes (CD11b+Gr1-). Naïve ($CD44^{lo}$) and memory ($CD44^{high}$) CD4+ (GK1.5, eBioscience, California, USA) and CD8+ (53–6.7, Southern Biotech, Alabama, USA) T cells were distinguished with the surface protein CD44 (1M7, eBioscience, California, USA). After staining, cells were washed and acquired on a BD C6 Accuri cytometer (BD Biosciences, California, USA). Gating strategies used to determine cell percentages are detailed in Fig. S1. The intensity of CD127 surface protein expression was assessed with an antibody to CD127 (A7R34, eBioscience, California, USA). The total number of naïve ($CD44^{lo}$) and memory ($CD44^{hi}$) CD4+ and CD8+T cells within the spleen and lymph node was calculated by multiplying the percentage of each subpopulation by the total number of cells in each organ. Replicates from at least two independent experiments were pooled for this analysis. The total number of replicates (combined from each independent experiment) are as follows: n=10, spleen and thymus AL; n=14 spleen and thymus MAL; n=7, lymph node AL; n=5, lymph node MAL.

2.3. Taqman quantitative reverse transcriptase PCR

After one week of malnourishment or control diet, the lymph nodes of three AL and 9 MAL mice were isolated. Given the significantly smaller size of the lymph nodes from MAL mice, the lymph nodes from 3 individual MAL mice were pooled. RNA was isolated, DNased, and reverse transcribed with the FastSCRIPT cDNA Synthesis Kit (Tonbo). The cDNA from each sample was subjected to quantitative PCR specific to the IL-7 and β-2 microglobulin genes using the FastPROBE qPCR Hi-ROX Master Mix (Tonbo). IL-7 was targeted with primers and probes included in the PrimeTime Redesigned qPCR assay (Mm. PT.58.10325839, Integrated DNA Technologies) and β2 microglobulin was targeted with the following primers and probe:

5′-/56-FAM/TTC AAG TAT /ZEN/ACT CAC GCC ACC CAC C/3IABkFQ/-3′; primer 1: 5′-GGT CTT TCT GGT GCT TGT CT-3′; primer 2: 5′-ACG TAG CAG TTC AGT ATG TTC G-3′ (Integrated DNA Technologies). Note that "ZEN" refers to an additional quencher placed 9 bases from the fluorophore to reduce background fluorescence.

2.4. CD8+T cell isolation, culture, and death-by-neglect

After 6 weeks of the dieting regimen, CD8+T cells were enriched from the spleens and lymph nodes of male and female

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