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Differential IL-7 responses in developing human thymocytes

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ABSTRACT

Interleukin (IL)-7 is a factor essential for mouse and human thymopoiesis. Mouse thymocytes have altered sensitivities to IL-7 at different developmental stages. CD4/CD8 double positive (DP) mouse thymocytes are shielded from the influence of IL-7 because of loss of CD127 (IL-7Rα). In this study, we assessed IL-7 receptor expression and IL-7 signaling in human thymocytes. We found human DP cells to be severely limited in their ability to phosphorylate STAT-5 in response to IL-7. The relative expression levels of the IL-7-inducible proteins Bcl-2 and Mcl-1 were also lower in human DP cells, consistent with a stage-specific decrease in IL-7 responsiveness. IL-7 responses were restored in a subset of cells that matured past the DP stage. Unlike the regulation of IL-7 signaling in mouse thymocytes, loss of IL-7 signaling in human DP cells was not due to absence of CD127, but instead correlated with downregulation of CD132 (common γ chain).

1. Introduction

As thymocytes develop from early thymic progenitors to mature single positive T cells (SP4 or SP8), they differentially express many surface antigens that can be used to discriminate discreet subsets. Human and mouse thymocyte compartments have historically been discriminated using CD4 and CD8 expression as the principal markers, along with numerous other antigens to further divide the subpopulations [1–3]. Early thymocytes are negative for CD4 and CD8 as well as negative for T-cell antigen receptors (TCR) and its signaling partner CD3. After cells mature past β selection, they express both CD4 and CD8 (double positive [DP] cells), and eventually mature into cells that are single positive for CD4 (SP4) or CD8 (SP8) and express high levels of TCR and CD3.

It is evident in the early subsets that human thymocyte development differs from that of the mouse. For example, in humans, a thymocyte subset exists that is negative for CD3 and CD8, but positive for CD4. This population is an immature (ISP4) subset that is absent or very small in mice [4–6]. Therefore, CD3 or TCR expression, in addition to CD4 and CD8 expression, has historically been used to discriminate the least mature human thymocyte populations. These immature human thymocytes are referred to as the triple negative (TN) population (negative for CD3/TCR, CD4, and CD8) [1,7]. In mice, the least mature thymocytes are referred to as the double negative population; those that do not express CD4 or CD8. Different markers are often used for mouse cells compared with human cells to further divide the early pre-β-selection and post-β-selection thymocyte subsets [8–10], making direct comparisons of major selection events and minor subsets between the two species difficult.

Although mice and humans have incongruous patterns of expression of multiple developmental markers, Interleukin (IL)-7 signaling is indispensable for thymopoiesis in both of these mammalian systems. Mice genetically engineered to be deficient in expression of IL-7 or IL-7Rα have severely reduced numbers of thymocytes [11,12]. Likewise, disruptions or mutations in the human IL-7 signaling pathway lead to severe combined human immunodeficiency [13]. In severe combined human immunodeficiency, lack of a functioning immune system leads to systemic infections and ultimately patient death.

Despite an absolute requirement for IL-7 signaling in both human and mouse thymocyte development, surprising differences in IL-7 receptor expression patterns occur between the two species. It has been reported that human DP thymocytes express IL-7Rα [14], whereas, we and others have found mouse DP cells to lack IL-7Rα [6,15]. In mice, IL-7 signaling is finely tuned and differentially regulated during thymocyte development [15,16]. Although IL-7 signaling is required for early proliferation and maturation, excess IL-7Rα expression can be detrimental and leads to reduced mouse thymocyte numbers [16]. In normal mouse thymopoiesis, IL-7 sig-

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naling is shut off during the DP stage as cells audition for positive selection. This is accomplished by downregulation of IL-7Rα expression as well as cell migration into the thymic cortex where little IL-7 is expressed [17]. After positive selection, IL-7Rα is re-expressed and cells then enter the IL-7-rich medulla where IL-7-mediated survival signals can be turned on [15]. It has been hypothesized that the shutoff of IL-7 signaling in the murine DP compartment is a mechanism by which death-by-neglect of thymocytes that fail to successfully express a TCR can be enforced [16]. It has not been established previously to what extent similar control of IL-7 signaling occurs in humans.

In this study, expression of the IL-7 receptor chains (CD127 and CD132) and IL-7 responsiveness of human thymocyte subsets were measured. Despite marked differences in IL-7 receptor expression compared with mice, human thymocyte subsets were similar to mouse thymocytes in IL-7 responses. Human DP cells showed greatly diminished responses to IL-7 compared with the other compartments, as measured by STAT-5 phosphorylation which correlated with downregulation of CD132 in this subset. Additionally, expression of Bcl-2 and Mcl-1 (IL-7-induced proteins) correlated with IL-7 sensitivity, suggesting loss of IL-7 signaling occurs *in vivo* at the DP stage of human thymocyte development.

2. Subjects and methods

2.1. Thymocyte isolation

Human thymus tissue was obtained during pediatric cardiac surgeries at Saint Francis Hospital in Tulsa, OK, and the Children's Hospital at OU Medical Center, Oklahoma City, OK, from patients aged 1 week to 3 years (mean age = 18.75 months). This was done in accordance with protocols approved by the Institutional Review Boards of both the University of Oklahoma and Saint Francis Hospital. Only freshly isolated thymocyte samples were used for the experiments in this report. This was necessary because of reported preferential death of early human thymocyte subsets in short-term (4°C) or long-term storage (−80°C or liquid nitrogen) [14]. Single-cell thymocyte suspensions were generated by forceful disruption of thymuses with 3 ml syringe plungers through 70-μm nylon screens. Thymocyte manipulations were conducted in complete tumor medium [18].

2.2. Antibodies and flow cytometry

Human thymocyte subpopulations were discriminated using fluorescein-isothiocyanate-coupled anti-CD3 mAb (Clone S4.1, Invitrogen/Caltag, Carlsbad, CA), Pacific Blue coupled anti-CD4 mAb (Clone RPA-T4, BioLegend, San Diego, CA), allophycocyanin/Cy7-coupled anti-CD8 mAb (Clone RPA-T8, BioLegend) and when sorting, a PE-coupled non-T lineage cocktail which included: CD11c (Clone 3.9, eBioscience, San Diego, CA), CD14 (Clone 61D3, eBioscience), CD19 (Clone H1B19, eBioscience), CD56 (Clone MEM188, eBioscience), and CD235a, glycophorin A (Clone H1R2, eBioscience). IL-7Rα expression was assessed using PE-labeled mAb specific for CD127 (Clone 40,131, R&D Systems, Minneapolis, MN), and PE-coupled mouse IgG_1 (Clone 11,711, BD Pharmingen, San Jose, CA) was used as an isotype control. Common γ receptor expression was assessed using PE-labeled mAb specific for CD132 (Clone TUGh4, BioLegend) and a PE-coupled rat IgG2b, κ (BioLegend) was used as an isotype control. Cells were analyzed using an LSR II Special Order System flow cytometer (BD Biosciences, San Jose, CA).

2.3. Intracellular pSTAT-5 staining

Fresh human thymocytes were sorted using a Dako MoFlo cell sorter (Carpinteria, CA). Before treatment with cytokine, thymocyte populations were incubated at 37°C for 20 minutes in warm S-MEM (Invitrogen, Carlsbad, CA). The cells were then incubated in either S-MEM alone or with 25 ng/ml human IL-7 (R&D Systems, Minneapolis, MN) for an additional 20 minutes. At the end of the incubation, cells were fixed and stained for intracellular pSTAT-5 (Tyr^{694}) (BD Biosciences) as described previously [16,19].

2.4. Western blotting

Thymocyte subsets were sort purified and NP-40 whole cell lysates were generated from untreated and cytokine-treated (25 ng/ml IL-7 or interferon-γ) samples [15,19] and then Western blotted as described previously [18,20], using primary antibodies specific to phosphorylated STAT-5 (Cell Signaling; rabbit polyclonal against human), Mcl-1 (BioVision, Mountain View, CA; rabbit polyclonal against human), Bcl-2 (Santa Cruz Biotechnology, Inc.; mouse monoclonal against human), and β-actin (Santa Cruz Biotechnology, Inc.; goat polyclonal against human). Horseradish peroxidase-conjugated secondary antibodies were polyclonal goat anti-rabbit IgG (KPL, Inc, Gaithersburg, MD), goat anti-mouse IgG (Santa Cruz Biotechnology, Inc.), and donkey anti-goat IgG (Santa Cruz Biotechnology, Inc.).

2.5. Data analysis

Flow cytometric data were analyzed for Table 1 using FACSDiva software (BD Biosciences). The histogram overlays shown in Fig. 1 were generated using FloJo software (Tree Star, Inc., Ashland, OR). Paired Student's *t* tests were performed using GraphPad Prism software to generate the *p*-values for the comparisons described in the Results (Section 3.1).

3. Results

3.1. IL-7Rα signaling is inhibited in DP thymocytes

Human thymocytes were stained with mAbs to CD3, CD4, CD8, CD127 (IL-7Rα), and CD132 (common γ receptor), as well as assessed for responses to IL-7 as described in the "Subjects and Methods." Populations were defined as follows: TN ($CD3^-$, $CD4^-$, $CD8^-$), ISP4 ($CD3^-$, $CD8^-$, $CD4^+$), DP^{CD3lo} ($CD3^{-/lo}$, $CD4^+$, $CD8^+$), DP^{CD3hi} ($CD3^+$, $CD4^+$, $CD8^+$), SP4 ($CD4^+$, $CD8^-$, $CD3^+$), SP8 ($CD8^+$, $CD4^-$,

Table 1
IL-7 receptor expression and IL-7 response profiles of human thymocytes

Patient (age/gender)	TN	ISP4	DP^{CD3lo}	DP^{CD3hi}	SP4	SP8
1 (2 yr/f)						
pSTAT5[a]	47.9	68.1	0.7	0.0	57.7	31.2
CD127[b]	55.6	99.1	93.3	77.6	86.7	89.2
CD132[b]	61.3	83.0	8.8	16.9	96.1	99.0
2 (6 mo/m)						
pSTAT5[a]	59.3	46.2	0.0	1.3	61.2	29.9
CD127[b]	90.6	94.8	98.4	96.0	88.4	97.1
CD132[b]	97.2	94.8	13.5	27.3	98.9	99.9
3 (1 wk/f)						
pSTAT5[a]	35.4	65.9	0.4	0.5	42.2	7.0
CD127[b]	57.0	96.9	87.3	61.3	72.6	81.0
CD132[b]	78.7	75.4	3.1	9.5	85.1	89.8
4 (5 mo/f)						
pSTAT5[a]	48.2	24.9	0.3	1.0	58.1	26.5
CD127[b]	76.0	96.3	69.2	28.9	73.6	80.0
CD132[b]	58.1	41.7	1.5	8.1	82.6	84.7
5 (3 yr/f)						
pSTAT5[a]	23.2	19.0	0.9	0.0	52.6	29.4
CD127[b]	54.0	79.4	95.5	64.0	86.2	42.0
6 (3 yr/m)						
pSTAT5[a]	41.9	17.5	0.9	0.5	55.5	30.6
CD127[b]	90.3	64.0	99.0	81.2	79.1	53.5
7 (2 yr/f)						
pSTAT5[a]	50.4	16.5	0.1	0.2	52.1	17.5
CD127[b]	19.2	95.5	92.7	74.5	77.8	71.1

[a]Numerical values represent the percent staining positive for pSTAT5 in each IL-7-treated population relative to the corresponding untreated population.
[b]Numerical values represent the percent staining positive for surface expression of the indicated IL-7 receptor chain relative to isotype control staining.