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Detection of a novel population of fetal thymocytes characterized by preferential emigration and a TCRγδ+ T cell fate after dioxin exposure

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Abstract

T cell maturation into TCRαβ$^+$ or TCRγδ$^+$ cells from common immature CD4$^-$CD8$^-$ (DN) precursors occurs in the thymus, and is controlled through ordered regulation of genes. The aryl hydrocarbon receptor (AHR), a latent cytoplasmic transcription factor, affects thymocyte maturation and differentiation at several stages, also including DN cells. We analyzed in murine fetal thymus organ cultures (FTOC) the outcome of AHR-signaling and found a higher frequency of DN TCRγδ$^+$ cells in the presence of the AHR-activating ligand TCDD. We detected a novel population of CD25$^{int/lo}$CD44^{hi} cells associated with preferential emigration and a TCRγδ$^+$ T cell fate of thymocytes. Sorted DN TCRγδ$^+$ emigrants could proliferate if IL-2 was available. Moreover, they suppressed the proliferation of co-cultivated, activated CD4$^+$ T cells. Gene expression profiles of purified DN emigrants from TCDD*FTOC revealed 295 modulated genes, 10% of which are genes of the immune system. For instance, RAG-1, TdT, and Gfi-1 were downregulated, yet genes indicative of mature thymocytes were upregulated. In conclusion, we have detected changes in the differentiation programme of fetal DN thymocytes after ligand-activation of the AHR. In particular, we observed a higher frequency of DN TCRγδ$^+$ cells with high emigration potential, and possible regulatory functions.

Keywords: Thymus; Lineage commitment; Emigration; TCDD; Arylhydrocarbon receptor

Abbreviations: AHR, aryl hydrocarbon receptor; DN, CD4$^-$CD8$^-$ double negative; DP, CD4$^+$CD8$^+$ double positive; DRE, dioxin-responsive element; FTOC, fetal thymus organ culture; SP, single positive; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDD*FTOC, FTOC treated with TCDD.

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1. Introduction

T cells develop in the thymus from precursors along a tightly controlled, sequential differentiation pathway. Timing and control of lineage commitment towards the two functionally distinct T cell subsets, bearing either the TCRαβ or γδ, are not yet clear. The earliest precursor cells in the thymus are c-kit$^+$CD44$^+$ CD25$^-$CD4$^-$CD8$^-$ (DN1[4] cells). They upregulate CD25 to become c-kit$^+$CD44$^+$CD25$^+$CD4$^-$CD8$^-$ (DN2) cells. DN2 cells are still capable of developing into thymic dendritic cells and, albeit with poor efficiency, into NK cells [1,2]. Rearrangement of the γ,δ, or the β TCR genes, and thus the commitment to the T cell lineage, starts at the transition from the DN2 to the DN3 stage [3,4]. DN2 cells can be divided into IL-7R$^-$ and IL-7R$^+$ cells. The latter have a higher potential towards TCRγδ$^+$ cells, and TCRγδ$^+$ cells are dependent on IL-7/IL-7R and its intracellular signaling by Jak3 [3,5–7]. Rearranged β chains can be found in TCRγδ cells, and rearrangement of the TCRγ and TCRδ loci is found in most TCRαβ cells [8], (i.e. lineage decision seems to be independent of TCR expression). Yet, rearrangement and expression of the wrong TCR in finally committed cells leads to apoptotic cell death [3,9]. Apparently, the αβ and γδ precursors are not sequentially related, but seem to be alternatives, first developing side by side, then finally separating. Signaling via Jak3 is critical for development of TCRαβ as well as TCRγδ cells, but is continuously required for the maintenance of the γδ T cell lineage [6]. Additionally, a bias for the αβ T cell lineage is in part due to compromised survival and expansion of TCRγδ$^+$ cells, apparently caused by bcl-2 deficiency [7]. In contrast to the adult thymus, TCRγδ$^+$ cells predominate in the fetal thymus; the shift from pre- to post-natal development is characterized by successive waves of distinct Vγ genes used in rearrangement. The regulation of these developmental changes are not quite clear, albeit Notch1 is essential during a defined developmental window [10].

The AHR is a latent ligand-activated transcription factor, which is abundant in the thymus, thymus stromal cells, and many other tissues [11,12]. The AHR is an orphan receptor of the basic helix–loop–helix Per/ARNT/Sim homology (PAS-bHLH) family, whose members are involved in e.g. rhythm coordination, neurogenesis, and lineage specific transcription [11]. Similar to steroid receptors and many orphan receptors, the AHR becomes activated by specific low molecular weight ligands. A well-studied function of the AHR is ligand-dependent induction of the enzymes, which catalyze oxidative biotransformation of non-polar substances, such as aromatic hydrocarbons. The most efficient AHR-activating ligand is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a planar aromatic hydrocarbon, which has been extensively used to study the receptor and its toxicological implications. TCDD is produced accidentally during industrial processes, including the production of Agent Orange, the herbicide used during the Vietnam War. TCDD mediates its many toxic effects, including cancer, via the AHR. It is still considered a pollutant and health risk of considerable concern. Observations in AHR-deficient mice suggested a role for the AHR in the development of the immune system, the liver, vascular remodeling, and aging processes [13]. The large number of AHR target genes, which are involved in cell differentiation, cell cycling, cell activation, and the recent identification of possible endogenous ligands [14–17] support a role for the AHR in cell functions not connected to its well-known role in biotransformation of external substances.

Whether or not a gene becomes a direct target of the activated AHR is dependent on the presence of so-called DREs in its promoter, and on cell type and cell differentiation stage, i.e., overall accessibility of a particular locus [18]. In addition activation of the AHR can directly target genes via secondary effects. Genes inducible, whether directly or indirectly, by the AHR in thymocytes are, among others, Notch-1, IL-2, bcl-2, CD44 and adseverin [19–22] all of which are known for their role in thymocyte differentiation and survival.

Activation of the AHR changes thymocyte differentiation pathways at several checkpoints [23–25]. As early as 1992, a preferential generation of TCRγδ$^+$ cells was noted in thymocytes of fetuses treated in utero with an AHR ligand; a block at the transition phase from CD4$^-$CD8$^+$CD24$^+$ to CD4$^+$CD8$^+$ was suggested [26]. Further studies showed that in particular the proliferation of CD24$^+$ DN3 and DN4 thymocytes is diminished within 9–12 h after injection of TCDD into C57BL/6 mice, probably causing the well-known thymus atrophy within 10 days [27]. We