Available online at www.sciencedirect.com
SCIENCE DIRECT
Immunology Letters 104 (2006) 110–117
Immunology Letters
www.elsevier.com/locate/

Induction of chemokine receptor expression during early stages of T cell development

Sarah Maerki, Rod Ceredig, Antonius Rolink *

Developmental and Molecular Immunology, Department of Clinical and Biological Sciences (DKBW), University of Basel, Mattenstrasse 28, CH-4058 Basel, Switzerland

Received 29 October 2005; received in revised form 29 October 2005; accepted 8 November 2005
Available online 1 December 2005

Abstract

The early events in T lineage commitment are difficult to study because of the rarity of these cells. We have therefore used cloned Pax5$^{-/-}$ pre-BI cell lines as a model system to study this. Stimulation in vitro of Pax5$^{-/-}$ pre-BI cells with stromal cells expressing the Notch ligand Delta-like 1 results in them coincidently undergoing some of the phenotypic and functional changes associated with early T cell commitment. Kinetic analysis indicated that there was a rapid induction of transcripts for the two chemokine receptors CCR4 and CXCR6. Transcripts for CCR8 increased with slower kinetics. Migration assays indicated that Delta-like 1 signalling of Pax5$^{-/-}$ pre-BI cells had induced responsiveness to the chemokines MDC and MIP-1β, ligands for the receptors CCR4 and CCR8, respectively. Importantly, following Delta-like 1 signalling, similar increases in chemokine receptor transcripts were seen in a recently described bone marrow progenitor subpopulation having significant T cell progenitor activity and being phenotypically and functionally similar to Pax5$^{-/-}$ pre-BI cells. The relevance of these findings to studies of early T cell development will be discussed.

Keywords: Notch; T cells; Pax5$^{-/-}$ pre-BI cells; Chemokines; Chemokine receptors

1. Introduction

Cells of the hematopoietic system are continuously generated from hematopoietic stem cells (HSCs). HSCs reside in the bone marrow which is also the site of B lymphocyte development [1]. In contrast to B cells, T cell development takes place in the thymus which does not itself contain self-renewing HSCs and which therefore needs to be continuously repopulated by small numbers of progenitors migrating from the bone marrow [2,3]. In this process of migration, T progenitor cells have to be guided to their correct destination in the thymus [4]. So far, the signals involved in T cell progenitor migration are not known but chemokines and their receptors have been implicated in this process because blocking of $G_{\alpha i}$ protein coupled receptor signalling (which includes chemokine receptor signalling) leads to the inhibition of thymic seeding by precursor cells in vitro [5].

Notch ligands and their corresponding receptors are known to be involved in lineage commitment of developing hematopoietic cells [6] and the Notch1 receptor is known to be essential for T cell lineage commitment [7,8]. There is an ongoing discussion concerning the anatomic site where T cell progenitors first make contact with Notch ligands [9–11]. The current accepted model is that progenitors only receive these signals upon entry into the thymus [12]. In agreement with this, Notch ligands are indeed abundantly expressed at the site of progenitor entry [13]. However, the Notch ligand Delta-like 1 has also been shown to be expressed by bone marrow stromal cells [14]. Thus, the bone marrow could be involved in early stages of T cell lineage commitment. Notch signal strength and duration are also critical parameters in hematopoietic lineage commitment [15]. It has been suggested that progenitors may lose B cell lineage potential in response to low levels of Notch activation but are unable to undergo further T cell lineage differentiation before thymic entry [16]. In addition other signals, for example, cytokines, provided by the bone marrow microenvironment might also be involved in progenitor cell commitment along hematopoietic lineages [17].

Abbreviations: DL1, Delta-like 1; FSC, forward light scatter; HSC, hematopoietic stem cell; mAb, monoclonal antibody; SSC, side scatter

* Corresponding author. Tel.: +61 267 1631; fax: +61 695 3070.
E-mail address: Antonius.rolink@unibas.ch (A. Rolink).

0165-2478/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.imlet.2005.11.002

B cell development is dependent upon expression of the transcription factor Pax5 [18]. Deletion of the Pax5 gene results in a complete block of B cell development at the pre-BI stage [19]. When cultured on bone marrow stromal cell lines and in the presence of IL-7, freshly isolated pre-BI cells from $Pax5^{-/-}$ mice can be cloned and maintained in vitro for long periods of time retaining their pre-BI phenotype [18]. Although completely blocked in B cell differentiation, if stimulated with the appropriate combination of cytokines and stromal cells, $Pax5^{-/-}$ pre-BI cells can nevertheless differentiate in vitro along multiple hematopoietic lineages including T cells [18]. Thus, $Pax5^{-/-}$ pre-BI cells could be regarded as representatives of uncommitted progenitors that cannot proceed along the B cell lineage, their default developmental pathway. Indeed, we have recently described in the bone marrow of normal mice a rare cell sharing the phenotypic and functional properties of $Pax5^{-/-}$ pre-BI cells [20].

All efforts to generate T cells from progenitors in vitro in the absence of a thymic microenvironment had for a long time been unsuccessful. Recently, however, it was shown that progenitors cultured on OP9 stromal cells (OP9) ectopically expressing the Delta-like 1 molecule (OP9-DL1) and in the presence of the cytokine IL-7 could differentiate along the T cell lineage [21]. We have shown that this culture system has an extremely high plating efficiency for T cell progenitors [20,22]. Interestingly, $Pax5^{-/-}$ pre-BI cells were also shown to differentiate into T cells under these culture conditions [23]. Thus, the $Pax5^{-/-}$ pre-BI cells may provide a model system for investigating early events in T lineage commitment.

Our preliminary experiments had indicated that the levels of chemokine receptor transcripts were altered in $Pax5^{-/-}$ pre-BI cells upon culture on OP9-DL1. Therefore, we decided to investigate this in more detail. Results obtained show that coincident with phenotypic changes corresponding to early T cell commitment, there was a significant increase in certain chemokine receptor transcripts. Kinetic experiments showed that in some cases these increases were very rapid. In addition, migration assays indicated that corresponding chemokine receptors were funcionally expressed. The implications of these results in the context of T cell progenitor migration to the thymus will be discussed.

2. Materials and methods

2.1. Cells and cell culture

Cloned $Pax5^{-/-}$ pre-BI cells were cultured in Iscove's modified Dulbecco's medium (IMDM) supplemented with 5×10^{-5} M β-mercaptoethanol, 1 mM glutamine, 0.03‰ (w/v) Primatone (Quest, Naarden, NL), 100 U/ml penicillin, 100 μg/ml streptomycin and with 2% FCS, 5% of IL-7-conditioned medium and 0.5% Ciproxin® 0.2 g (10 μg/ml final concentration) on a confluent layer of γ-irradiated (3000 rad) OP9 or OP9-DL1 stromal cells in a humidified incubator at 37 °C and 10% CO_2. Culture of stromal cell lines was performed as previously described [20]. The mean doubling time of $Pax5^{-/-}$ pre-BI cells was about 24 h and cultures were split every 3–4 days. For transfer, cells were mechanically dispersed to detach the pre-BI cells from the stromal cell layer. $Pax5^{-/-}$ pre-BI cells were seeded into 75 cm^2 flasks on a pre-prepared confluent layer of stromal cells at pre-determined concentrations such that cell recovery was optimal at the desired time point. Transfer of cells from OP9 to OP9-DL1 stroma resulted in a transient delay in cell proliferation.

2.2. Isolation of bone marrow progenitor cells

Bone marrow cell suspensions from two femurs and two tibias were collected, washed and erythrocytes lysed using 0.1 M ammonium chloride. Washed cells were stained for 20 min on ice with a combination of $CD45R^{FITC}$, $CD3^{PE}$, $CD19^{PE}$, $NK1.1^{PE}$, $CD117^{APC}$, $CD93^{bio}$, washed and biotin-labelled CD93 was revealed with streptavidin-PE-Cy7. Washed and filtered cells at a concentration of 20×10^6 ml^{-1} were sorted on a FACSAria (Becton Dickinson) into $CD45R^+$, $CD117^{low}$, $CD3^-$, $CD19^-$, $NK1.1^-$, $CD93^+$ cells as recently described [20].

2.3. Antibodies for FACS analysis

The following mAb were purchased from PharMingen (San Diego, USA): $CD45R^{FITC}$ (RA3-6B2), $CD25^{FITC}$ (7D4), $CD3\varepsilon^{PE}$ (145.2C11), $CD19^{PE}$ (1D3) and $NK1.1^{PE}$ (PK136). The $CD117^{APC}$ (2B8) mAb was purchased from eBioscience (San Diego, USA). Anti-CD93 (PB493) antibody was purified from the hybridoma and labelled with biotin in our laboratory according to standard techniques. As secondary FACS reagents streptavidin-PE-Cy7 (PharMingen) and streptavidin-PE (Southern Biotech, Southern CA, USA) were used.

2.4. Flow cytometry

Cultured cells detached from the stroma were adjusted to $10–20 \times 10^6$ cells/ml in PBS supplemented with 2% FCS and 0.2% azide. For surface staining, $(0.5–1) \times 10^6$ cells were incubated for 30 min on ice with the indicated reagents at saturating concentrations. Following a washing step, biotin-labelled antibodies were revealed by incubation with streptavidin-PE or streptavidin-PE-Cy7 for 15 min on ice. Stained cells were analyzed using a FACSCalibur (Becton Dickinson) and data was analyzed using CellQuest Pro Software (Becton Dickinson). Viable cells were defined by a combination of FSC, SSC and propidium iodide exclusion. Intracytoplasmic staining of permeabilized fixed cells was carried out by standard procedures [20].

2.5. Measurements of gene expression

RNA was prepared from 10^4 to 10^7 cells using the TriREAGENT-Kit (Molecular Research Center, Inc., Cat. No. TR-118) as indicated by the manufacturer. The final RNA concentration was then measured by absorption and the adequate amount of RNA was used for cDNA synthesis and the remaining RNA was stored at −80 °C. To remove possible contaminating DNA, samples were treated with RQ1 RNase-free DNase 1 U/μl (Promega, M610A). cDNA was