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Temporal regulation of Stat5 activity in determination of cell differentiation program

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Abstract

Although Stat5 is activated by various cytokines, only erythropoietin (Epo) and a small number of cytokines induce Stat5-dependent erythroid differentiation. Here, by using a reporter gene system to monitor transcriptional activity of Stat5, we showed that Epo but not interleukin (IL)-3 supports sustained activation of Stat5, which induces globin gene expression. IL-3 or IL-2 stimulation inhibits Epo-induced globin gene expression. The acidic region of the IL-2 receptor β chain was essential for this inhibition. These results underscore the importance of temporal regulation of Stat activity for regulation of cytokine-specific cell differentiation.

Keywords

erythropoietin; IL-3; IL-2; globin genes; transcription; erythroid differentiation

Introduction

Epo plays essential roles in erythropoiesis [1], in which Stat5 activation by Epo [2] is important [3-8]. Although Stat5 is activated by a variety of cytokines [2], only Epo and small number of other cytokines induce erythrocyte differentiation *in vivo* and *in vitro* model systems [1,9]. It is not well understood how specificity of Stat5-mediated globin gene expression by Epo is ensured.

Here we showed that LG-Stat5, an active-form of Stat5, induces globin gene expression in response to epidermal growth factor (EGF) in EGF receptor (EGFR)-reconstituted Ba/F3 cells. LG-Stat5-induced globin gene expression is inhibited by IL-3, which activates Stat5 but does not induce globin gene expression. By using a reporter gene system, we showed that EGF and Epo stimulation induce sustained reporter gene expression by LG-Stat5, whereas IL-3-induced reporter gene expression is downregulated gradually. These results indicate that sustained activation of Stat5 mediates erythroid differentiation and globin gene expression. Furthermore, we showed that IL-2 also mediates inhibition of Epo-induced globin gene expression, for which the acidic region (the A-region) of the IL-2 receptor (IL-2) β chain (IL-2R β) is essential for this inhibition. These results underscore the importance of temporal regulation of Stat activity for regulation of cytokine-specific gene expression and may provide insight into mechanism of crosstalk in cytokine signaling.

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Materials and Methods

Plasmids and gene transfer

Plasmid construction and gene transfer are described in Supplemental Methods.

Immunoblot analysis

Preparation of nuclear extracts and immunoblot analysis were described previously [10].

Inducible translocation trap (ITT) assay and flowcytometry

For ITT assay, BBLG or BLG transduced with pLG-Stat5 was analyzed for green fluorescent protein (GFP) with FACS Calibur as described previously [10].

DNA microarray analysis

BBLG expressing LG-Stat5 was cultured in the presence of mouse IL-3 (1 ng/ml) or human EGF (10 ng/ml). Gene expression was analyzed using Affymetrix expression array with GeneChip Murine Genome set U74Av2 according to the manufacturer's instruction with some modification.

Northern blot analysis

Northern blot analysis was performed as previously described [11]. cDNA probes of mouse α - and β -globin genes are described in Supplemental Methods.

Results and Discussion

EGF induces sustained reporter gene expression by an active form of Stat5

To analyze nuclear translocation and target gene expression by Stat5 using ITT [10], we transduced a retroviral vector encoding a fusion protein consisting of LexA DNA-binding domain (DB), transactivation domain of Gal4 (Gal4 TA), and Stat5 (LG-Stat5) (Fig. 1A) into BBLG cell line containing LexA-d1EGFP reporter gene in the genome [10]. BBLG was derived from BB13, which was derived from murine IL-3-dependent hematopoietic Ba/F3 and ectopically expresses EGFR and Bcl-2 [12]. EGF supports proliferation of BB13 and BBLG but not parental Ba/F3. When LG-Stat5 is activated and translocates into the nucleus, it binds to LexA operator sites of the GFP reporter gene as well as Stat-binding elements of its target genes (Fig. 1B). In addition, since LG-Stat5 has a strong TA of Gal4, LG-Stat5 can function as an active form (Fig. 1B, see below). After Transduction of LG-Stat5 into BBLG cells, GFP (+) cells were sorted and cultured in the presence of EGF and/or IL-3. GFP expression levels were very high just after sorting of GFP (+) cells (day 0, Fig. 1C, left panel). Expression levels of GFP in cells cultured in the presence of IL-3 markedly decreased by day 8 (Fig. 1C, upper right panel). In contrast, cells cultured in the presence of EGF retained high GFP expression (Fig. 1C, middle right panel). Cells cultured in the presence of both IL-3 and EGF showed similar expression levels of GFP to cells cultured in the presence of IL-3 (Fig. 1C, lower right panel), indicating that the effect of IL-3 is dominant over EGF.

Sustained activation of Stat5 induces globin gene expression

To examine the consequence of sustained activation of Stat5, we analyzed the genome-wide gene expression profile of BBLG expressing LG-Stat5 using DNA microarray analysis. Interestingly, EGF but not IL-3 stimulation induced expression of globin genes in BBLG expressing LG-Stat5 (Fig. 1D). We confirm expression of globin genes in EGF-stimulated but not IL-3-stimulated BBLG expressing LG-Stat5 by Northern blot analysis (Fig. 1E). Importantly, BBLG did not express globin genes in the absence of LG-Stat5 expression (Fig.

1E, lane 1), suggesting that LG-Stat5 is necessary for EGF-induced globin gene expression. Since overexpression of Stat5 did not support globin gene expression in BBLG stimulated with EGF (data not shown), enhanced transcriptional activity by Gal4 TA is necessary for EGF-induced globin gene expression. There was a shift of β -globin alleles away from heterochromatin regions within the nucleus of EGF-stimulated BBLG expressing LG-Stat5 as compared with IL-3-stimulated cells (H.F., Jane Skok, unpublished observation), further confirming that EGF-induced globin gene expression in BBLG expressing LG-Stat5 is regulated in a similar manner with primary cells [13].

Epo induces sustained activation of Stat5

Considering that sustained activation of Stat5 induced globin gene expression as shown above, we investigated whether Epo supports sustained activation of Stat5. LG-Stat5 was transduced in BLG expressing EpoR, and GFP reporter expression was examined in the presence of IL-3, Epo, or both IL-3 and Epo. BLG is a Ba/F3-derived cell line containing the LexA-d1EGFP reporter gene [10]. Epo but not IL-3 supported sustained expression of GFP 15 days after stimulation (Fig. 2A). Sustained expression of GFP reporter was not observed when cells were cultured in the presence of both IL-3 and Epo, suggesting that IL-3 dominantly suppresses sustained activation of Stat5. Sustained activation of Stat5 was well correlated with expression of globin genes [14-16] (Fig. 2B). These results showed that Epo induces sustained activation of Stat5 and suggest that the temporal regulation of Stat5 activity is critical for erythroid differentiation and globin gene induction. Since globin genes are not direct target of Stat5 [17], it is likely that Stat5 regulates expression of critical transcription factors directly involved in globin gene expression. The nuclear amounts, tyrosine-phosphorylation, and DNA-binding activity of Stat5 in Epo-stimulated cells were not more than those in IL-3-stimulated cells (data not shown), suggesting that the Epo supports sustained activation of Stat5 through other mechanisms.

IL-3-mediated inhibition of reporter gene expression is not caused by the inaccessibility of the reporter locus

To examine whether IL-3-mediated inhibition of reporter gene expression is caused by inaccessibility of the reporter locus caused by heterochromatinization or other mechanisms, we transduced LG-Smad3 [10] into BLG expressing LG-Stat5, in which GFP reporter expression was down-regulated by IL-3 (Fig. 3A). LG-Smad3 is a fusion protein of LexA DB, Gal4 TA and Smad3, which translocates into the nucleus in response to transforming growth factor- β (TGF β). TGF β stimulation markedly induced GFP expression in BLG expressing LG-Smad3 (Fig. 3B), indicating that LG-Smad3 can access to the reporter locus and that the IL-3 stimulation does not close GFP reporter locus.

The A-region of IL-2R β is essential for IL-2-mediated inhibition of Epo-induced globin gene expression

IL-2 and IL-3 induce activation of similar, but not identical, signaling events [2]. IL-2R consists of the α -chain (IL-2R α), IL-2R β , and the common cytokine receptor γ -chain (γ c) [2]. Ba/F3 cells express IL-2R α and γ c but not IL-2R β [18]. The cytoplasmic domain of IL-2R β consists of three subregions, the serine-rich (S)-, acidic (A)- and H-regions (Fig. 4A) [19]. It has been shown that the membrane-proximal S-region, which recruits the Jak1 protein tyrosine kinase, is essential to invoke receptor signaling [19]. To examine whether IL-2 also inhibits Epo-induced globin gene expression, we transduced retroviral vector expressing mouse IL-2R β WT into EpoR-reconstituted Ba/F3. Sorted IL-2R β (+) cells were stimulated with IL-2, Epo, or IL-2 + Epo for 5 days. Epo but not IL-2 induced expression of globin genes (Fig. 4B). In addition, IL-2 inhibited Epo-induced globin gene expression (Fig. 4B).

We transduced retroviral vectors expressing mouse IL-2R β mutants lacking the A-region (A-mutant), the H-region (H-mutant) [20], or most of the cytoplasmic region (ST-mutant) into Ba/F3. Sorted IL-2R β (+) cells were stimulated with Epo or IL-2 + Epo for 5 day. The ST-mutant could not inhibit Epo-induced globin gene expression, indicating that the cytoplasmic domain of IL-2R β is required for IL-2-mediated inhibition of globin gene expression (Fig. 4C). The H-mutant, which lacks the H-region but retains the A-region and does not activate Stat5/3, could inhibit globin gene expression by Epo (Fig. 4C), indicating that the H-region and IL-2-induced Stat activity are dispensable for the inhibition activity. In contrast, the A-mutant, which lacks the A-region, failed to inhibit globin gene expression induced by Epo (Fig. 4C). These results showed that the A-region of IL-2R β is essential for IL-2-mediated inhibition of Epo-induced globin gene expression and suggest a potential role of signaling molecules activated through the A-region in IL-2-mediated inhibition of Epo-induced globin gene expression. We examined whether the A-mutant, which lacks inhibitory signaling for globin gene expression, can mediate globin gene expression by IL-2. IL-2 did not induce globin gene expression in cells expressing the A-mutant of IL-2R β (Fig. 4D), suggesting that a signaling pathway(s) specifically induced by EpoR may be necessary to induce erythroid differentiation and globin gene expression. Alternatively, the A-region may mediate both inhibitory and stimulatory signaling events for globin gene expression. It is an important future issue which signaling molecule is involved in IL-3/IL-2-mediated inhibition of sustained activation of Stat5.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Figure 1. Sustained activation of Stat5 induces globin genes. (A) LG-Stat5 consists of LexA DB (a.a. 1-87), Gal4 TA (a.a. 768-881), and human Stat5a. (B) When LG-Stat5 translocates into the nucleus by cytokine stimulation inducing Stat5 activation, it binds to the LexA operators with LexA DB and activates GFP expression by the action of Gal4 TA. Activated LG-Stat5 also binds to recognition sequences of Stat5 and activates expression of Stat5 target genes. (C) EGF stimulation induces sustained reporter expression by an active form of Stat5. After transduction of LG-Stat5 into BBLG cells, GFP (+) cells were sorted and cultured in the presence of EGF and/or IL-3. Expression of GFP was examined just after sorting (Day 0) and 8 days after sorting (Day 8). (D) Total RNA prepared from BBLG expressing LG-Stat5 cultured in the presence of IL-3 or EGF were subjected to microarray analysis with GeneChip Murine Genome set U74Av2. For all expressed genes, the changes relative to IL-3-stimulated cells (x axis) or EGF-stimulated cells (y axis) are plotted on log₁₀ format. (E) Expression of globin genes in BBLG expressing LG-Stat5 cultured in the presence of IL-3, EGF, or IL-3 + EGF. BBLG was transduced with LG-Stat5, and sorted GFP (+) cells and BBLG were cultured with IL-3 (1 ng/ml), EGF (10 ng/ml), or IL-3 + EGF for 4 days.

Figure 2.

Epo induces sustained activation of Stat5 and globin gene expression. (A) Expression levels of the GFP reporter in BLG expressing EpoR and LG-Stat5. After transduction of LG-Stat5 into EpoR (+) BLG, GFP (+) cells were sorted and cultured in the presence of Epo, IL-3, or IL-3 + Epo. Expression of GFP was examined 15 days after sorting. (B) Expression of globin genes in BLG expressing EpoR. Cells were cultured in the presence of IL-3 (1 ng/ml), Epo (5 ng/ml), or IL-3 + Epo for 4 days.

Figure 3.

IL-3-mediated inhibition of reporter expression is not caused by the inaccessibility of the reporter locus. (A) Scheme of the assay to examine accessibility of the GFP reporter locus using LG-Smad3. (B) BLG expressing LG-Stat5 was cultured in the presence of IL-3 for 10 days. Then, cells were further transduced with LG-Smad3 and cloned by single cell sorting. Clones expressing LG-Smad3 were mock-stimulated (thin line) or stimulated with human TGF β (2 ng/ml) (thick line) for 4 hr. GFP expression was analyzed by flowcytometry.

Figure 4.

The A-region of IL-2Rβ is required for IL-2-mediated inhibition of Epo-induced globin gene expression. (A) Schematic view of mouse IL-2Rβ and its mutants. The H-, A-, and ST-mutants of mouse IL-2Rβ lack the H-region (a.a. 383-513), the A-region (a.a. 316-385), or most of the cytoplasmic domain (a.a. 272-513), respectively. (B) IL-2 inhibits Epo-mediated globin gene expression. EpoR(+) Ba/F3 expressing WT IL-2Rβ were cultured with IL-2 (10 ng/ml), Epo (5 ng/ml), or IL-2 + Epo for 5 days. (C) The A-region of IL-2Rβ is required for IL-2-mediated inhibition of Epo-induced globin gene expression. EpoR(+) Ba/F3 expressing WT or mutants of IL-2Rβ were cultured with Epo or IL-2 + Epo for 5 days. (D) The A-mutant of IL-2Rβ failed to induce globin gene expression in response to IL-2. EpoR(+) Ba/F3 expressing the A-mutant of IL-2Rβ were cultured with IL-2 or Epo for 5 days.