Identification of DC21 as a Novel Target Gene Counter-regulated by IL-12 and IL-4

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Received 3 September 2002, Accepted 5 October 2002

The Th1 vs. Th2 balance is critical for the maintenance of immune homeostasis. Therefore, the genes that are selectively-regulated by the Th1 and Th2 cytokines are likely to play an important role in the Th1 and Th2 immune responses. In order to search for and identify the novel target genes that are differentially regulated by the Th1/Th2 cytokines, the human PBMC mRNAs differentially expressed upon the stimulation with IL-4 or IL-12, were screened by employing the differential display-polymerase chain reaction. Among a number of clones selected, DC21 was identified as a novel target gene that is regulated by IL-4 and IL-12. The DC21 gene expression was up-regulated either by IL-4 or IL-12, yet counter-regulated by co-treatment with IL-4 and IL-12. DC21 is a dendritic cell protein with an unknown function. The sequence analysis and conserved-domain search revealed that it has two AU-rich motifs in the 3'UTR, which is a target site for the regulation of mRNA stability by cytokines, and that it belongs to the N-acetyltransferase family. The induction of DC21 by IL-12 peaked around 8-12 h, and lasted until 24 h. LY294002 and SB203580 significantly suppressed the IL-12-induced DC21 gene expression, which implies that PI3K and p38/JNK are involved in the IL-12 signal transduction pathway that leads to the DC21 expression. Furthermore, tissue blot data indicated that DC21 is highly expressed in tissues with specialized-resident macrophages, such as the lung, liver, kidney, and placenta. Together, these data suggest a possible role for DC21 in the differentiation and maturation of dendritic cells regulated by IL-4 and IL-12.

Keywords: Counter-regulation, DC21, Interleukin-4, Interleukin-12, Th1 and Th2
dendritic cell activation and differentiation.

**Material and Methods**

**Cell culture** Mononuclear cells were isolated from freshly-drawn peripheral blood of healthy donors using Ficoll-Hypaque density gradient centrifugation. Typically, the mononuclear preparation from the peripheral blood contained 65-70% T cells, 15-20% B cells, and 10-15% monocytes. The cells were cultured in a RPMI media that contained 10% FBS as described (Koh et al. 2000). Recombinant human IL-4 and IL-12 (R + D systems, Minneapolis, USA) were treated to cells for the indicated time, and the cells were cultured in humidified 5% CO₂ at 37°C (Song et al., 2001).

**DD-PCR screening and cloning** PBMCs (1 × 10⁷) were cultured in the presence of IL-4 or IL-12 after which total cytoplasmic RNAs were isolated using 4 M GITC and 5 M CsCl through ultracentrifugation. DD-PCR was performed using a DD-PCR kit (RNAmapTM, GenHunter, Nashville, USA), according to the manufacturers instructions. The products were displayed on a 6% sequencing gel. The PCR products that were selected for the differential expression were purified from the gel, reamplified, and inserted into a pUC18/SmaI vector by ligation at 16°C for 48 h. After transformation, the clones that contained the insert were selected, and the plasmids were purified. Sequencing was then performed by using a T7 Sequencing kit (Park et al., 2000).

**RT-PCR** RT-PCR was performed using the following primers for DC21: 5' primer, gtctcttctgtcttgctcgtt; 3' primer, aaaaagatcaacag aagctct; β-actin: 5' primer caagagatggcaagccgtc; 3' primer tcctcttcgatcgcgctgca under the conditions described (Cho et al., 1999).

**Inhibitor assays** Purified PBMCs (2 × 10⁷/sample) were treated with the media alone, IL-12 (20 ng/ml) or together with 10 μM LY294002 (Sigma, St. Louis, USA), 200 nM wortmannin (Sigma), and 10 μM SB203580 (Calbiochem, Darmstadt, Germany). Inhibitors were treated to cells 1 h prior to the IL-12 addition as described (So et al., 2001). After a 12-h culture, the total RNAs were isolated and processed for RT-PCR with the specific DC21 primers.

**Tissue mRNA blot analysis** A multiple tissue mRNA blot membrane was purchased from Clontech (Palo Alto, USA), and a cDNA probe that represented nt #648 to #1060 of DC21 was labeled with [α-32P]dCTP (3,000 μCi/mmol; Amersham, Uppsala, Sweden) at the specific activity of 5 × 10⁶ cpm/mg and used for hybridization.

**Results and Discussion**

As an approach to search for novel genes that were selectively regulated by the Th1 and Th2 cytokine, freshly isolated human PBMCs were stimulated with IL-4 and IL-12, a prototypical Th2 and Th1 cytokine, respectively. The purified mRNAs were subjected to DD-PCR using oligo-dT and arbitrary primers, as described in Material and Methods. On the DD-PCR screen, several clones were selected for their differential expression pattern (Fig. 1). The candidates were subsequently cloned for sequencing. Through a GenBank data search, one DD-PCR product (clone A) was found to represent the 3'UTR of the *Homo sapience* dendritic cell protein DC21 (Xu, et al., 2001). For the full-length cDNA cloning, RT-PCR primers were designed, based on the cloned 3'UTR sequence and the reported 5' sequence of DC21. The RT-PCR product was 1.1 kb in size, corresponding to the size of the expected full cDNA, which was then confirmed to be DC21 by sequencing (Fig. 2). Human DC21 is a protein of 21 kDa that is composed of 192 amino acids. No function has been assigned to this protein. The nucleotide sequence analysis revealed the presence of AU-rich motifs in its 3'UTR, implicated in the rapid mRNA turnover by the action of mRNA-destabilizing factors (Shaw and Kamen, 1986). In addition, the C-terminal half of the coding region exhibited a high (70%) homology with the N-acetyltransferase domain that is found in several transcription factors (Soutoglou et al., 2001; Fry and Peterson, 2002) (Fig. 2).
Regulation of DC21 Gene Expression by IL-12 and IL-4

In order to understand the signaling mechanism that is involved in the DC21 induction, the effects of specific kinase inhibitors for the signaling pathways were then analyzed. IL-12 is reported to evoke PI3K, p38MAPK, and JNK pathways in monocytes/dendritic cells, which has been implicated in the activation and differentiation of these cells (Yang et al., 1998; Athie et al., 2000; Zhang and Kaplan, 2000). We observed that in human PBMCs, the expression reached its maximum level about 8-12 h after the IL-12 stimulation, which was maintained by 24 h at a comparable level (Fig. 4). The induction of DC21 by IL-4 displayed similar kinetics, and consistent results were observed for the counter-regulation of the DC21 expression upon the co-stimulation of IL-12 and IL-4 during 8-24 h (data not shown). This indicates the time-dependent regulation of the DC21 gene expression by the Th1 vs. Th2 cytokines in human primary immune cells. In contrast, several established-immune cell lines including THP1 and U937, DC21 levels were not subjected to regulation upon treatment with IL-12 or IL-4 (data not shown).

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Fig. 3. Counter-regulation of DC21 mRNA expression by IL-4 and IL-12. Purified PBMCs (2×10⁷/sample) were treated with IL-4 (5 ng/ml), IL-12 (20 ng/ml), or IL-4 plus IL-12, and cultured for 24 h in cRPMI. Total RNAs were isolated and processed for RT-PCR with DC21-specific primers that were designed as described in the text: 5’ primer: GTGCTTCTGATC TGTCAG 3’ primer; AAAAAAAAAAAGAGAAGCAGCTCT.

Fig. 4. Kinetics of DC21 mRNA induction by IL-12. Purified PBMCs (2×10⁷/sample) were treated with the media alone (lane 1) or IL-12 (20 ng/ml) for 2 h (lane 2), 4 h (lane 3), 8 h (lane 4), 12 h (lane 5), and 24 h (lane 6). The total culture time for all of the samples was 24 h. The RNAs were then isolated and processed for RT-PCR with specific primers of DC21, as in Figure 3.
effect at concentrations that are known to inhibit PI3K. In addition, SB203580, an inhibitor for both p38MAPK and JNK (Chen et al., 1998), abolished the IL-12-mediated up-regulation of the DC21 gene expression (Fig. 5). These data suggest that the LY294002-sensitive kinase and p38/JNK pathways are likely involved in the IL-12-induced up-regulation of DC21 in these cells. The differential sensitivity to LY294002 and wortmannin has been observed for a few other signal transduction pathways that are involved in a specific gene expression (Ramech et al., 1995; Adi et al., 2001). Based on these studies, it has been postulated that although LY294002 and wortmannin are both known to be specific inhibitors of the PI3K pathway, distinct down-stream kinases and effectors of PI3K (such as Akt and ribosomal S6 kinase) may be differentially affected by these two inhibitors.

Finally, in order to gain insight into the DC21 function, we analyzed the tissue-specific expression pattern of DC21 (Fig. 6). Results from the multiple-tissue blot with the DC21 cDNA probe demonstrate that the DC21 expression is very high in the kidney, liver, and lung, which represents tissues that are abundant with specialized-resident macrophages, such as mesangial cells, Kuffer cells, and alveolar macrophages, respectively. In particular, the highest expression was found in the placenta, which was recently reported to contain activated dendritic cell populations (Kammerer et al., 2000). As compared to PBLs, which contain monocytes and some macrophages that undergo differentiation, the lymphoid tissues (such as the thymus and spleen) exhibited a rather low expression of DC21. This suggests that DC21 tends to be expressed in mature differentiated-macrophage/dendritic cells when compared to monocytes or other immature immune cells. In addition, an analysis of the DC21 expression using several immune cell lines revealed that the myeloid lineage cells (e.g. U937) exhibited a relatively higher level of the DC21 expression than B or T lymphoid cells (data not shown). The presence of the acetyltransferase domain suggests the potential function of DC21 as a nuclear transcription factor that is involved in macrophage/dendritic cell differentiation, probably by histone acetylation and subsequent transcriptional regulation (Soutoglou et al., 2001; Fry and Peterson, 2002). In this regard, it is noteworthy that role of the histone acetylation in the Th1 vs. Th2 differentiation and cytokine gene expression has been recently suggested (Avni et al., 2002; Field et al., 2002). In order to demonstrate whether or not the expression and regulation of DC21 indeed occurs during the dendritic cell maturation and activation, studies are in progress that employ a dendritic cell culture derived from blood monocytes by IL-4 and GM-CSF treatments.

DC21 does not appear to be an abundant or house-keeping gene. This is evidenced by the fact that its cloning was only recently achieved upon the completion of the Human Genome Project. The identification of DC21 as a gene that is differentially regulated by IL-4 and IL-12 by DD-PCR in this study seemed possible due to the nature of its 3’UTR that possesses AU-rich motifs. This is a site for the regulation of mRNA stability that is found in transiently-inducible genes by growth factors, stress, and inflammation (Shaw and Kamen, 1986). Dendritic cells have recently emerged as a master regulator of the Th1 and Th2 immunity by differentiation into DC1 vs. DC2 subsets that determine the Th1 vs. Th2 differentiation (Viney, 1999; Lir et al., 2000; Tanaka et al., 2000; Moser and Murphy, 2002). Thus, further identification
of the genes that are involved in the Th1 vs. Th2 cytokine response in dendritic cells would provide valuable targets for deciphering the regulation mechanisms of the Th1 vs. Th2 immunity.

Acknowledgments This study was supported in part by grants that were provided through the Basic Science Research Program (KOSEF R01-19999-0000-00138-0) and the 21st Century Frontier Human Genome Research Program from MOST. K. A. Kong and J. Y. Jang were supported by the Brain Korea 21 Programs.

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