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# Regulation of Interleukin-2 Receptor $\gamma$ Chain mRNA Expression in Human Monocytic Cell Line THP-1

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The interleukin-2 receptor (IL-2R) $\gamma$ chain ($\gamma$c chain) is shared by IL-4R, IL-7R, IL-9R, and IL-15R and plays an important role in regulation of the immune system. However, its regulation in monocytic cell lines has not been well clarified. We examined the expression and regulation of the IL-2R$\alpha$, IL-2R$\beta$, $\gamma$c chain, IL-4R and IL-7R mRNA in a human monoblastic leukemia cell line, THP-1. Unstimulated THP-1 cells constitutively expressed a low level of $\gamma$c chain and IL-4R mRNA. Phorbol myristate acetate (PMA) induced macrophage-like differentiation and up-regulated the $\gamma$c chain mRNA expression in THP-1 cells. This effect of PMA was suppressed by the protein kinase inhibitors H-7 and staurosporine. PMA did not affect the expression of the other IL-R mRNAs examined. 1$\alpha$, 25(OH)$_2$D$_3$ and interferon-$\gamma$ also induced differentiation of THP-1 cells, but these reagents did not affect the expression of the IL-R mRNAs in THP-1 cells. These findings suggest that the expression of the $\gamma$c chain mRNA is regulated by the PMA-dependent pathway and is not associated with that of the other IL-R mRNAs.

**Key words:** IL-2R $\gamma$ chain, phorbol ester, monocyte, differentiation, protein kinase

The high-affinity interleukin-2 receptor (IL-2R) is composed of three genetically different subunits, the $\alpha$, $\beta$, and $\gamma$ chains, which are expressed or regulated independently (1). Recent studies showed that the $\gamma$ chain of IL-2R is shared by IL-4R (2, 3), IL-7R (4, 5), IL-9R (6, 7) and IL-15R (8) as a functional subunit and is thus designated as the common $\gamma$ chain ($\gamma$c chain) of cytokine receptors. Moreover, mutations of the $\gamma$c chain gene have been reported in patients with X-linked severe combined immune deficiency (9). These findings suggest that the $\gamma$c chain plays an important role in the regulation and development of the immune system and/or the hematopoietic system. It has been reported that interleukins which bind to the $\gamma$c chain-associated cytokine receptors have various effects on human peripheral blood monocytes and/or monocytic cell lines. Expression of the $\gamma$c chain in monocytes or monocytic cell lines has been reported by some investigators (10-16). According to these reports, human peripheral blood monocytes constitutively express the $\gamma$c chain (10, 11) and its mRNA (12) at a low level, but the results were variable in monocytic cell lines that were thought to be more immature than peripheral blood monocytes. Expression of the $\gamma$c chain gene has been detected in various myelomonocytic cell lines including U-937 and HL-60 by the reverse transcriptase-polymerase chain reaction (RT-PCR) (13) or by Northern blot hybridization (14, 15), but not in Mono Mac 6 (16) or in resting THP-1 cells (14, 15). Ohbo *et al.* showed that phorbol 12-myristate 13-acetate (PMA)-treated THP-1 cells expressed a significant amount of $\gamma$c chain mRNA (15). PMA is known to activate some protein kinases and to induce macrophage-like differentiation of THP-1 (17). However, the effects of PMA stimulation on the $\gamma$c chain-associated interleukin receptors and the effects of other differentiation inducers such as interferon (IFN)-$\gamma$ and 1$\alpha$, 25(OH)$_2$D$_3$ have not been fully clarified yet. In the present study, we examined the expression of $\gamma$c chain mRNA and the $\gamma$c chain-associated interleukin receptor subunits in a human monoblastic leukemia cell line, THP-1, and its regulation by various differentiation inducers and protein kinase inhibitors by RT-PCR.

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## Materials and Methods

***Reagents.*** PMA was purchased from Sigma (St. Louis, MO, USA) and dissolved in DMSO. H-7 and staurosporine were purchased from Seikagaku (Tokyo, Japan). 1$\alpha$, 25-dihydroxyvitamin $D_3$ (1$\alpha$, 25$(OH)_2D_3$) dissolved in ethanol was purchased from Calbiochem (La Jolla, CA, USA). Recombinant human IFN-$\gamma$ was purchased from PeproTech (Rocky Hill, NJ, USA). PCR primers for various cytokine receptor genes including the $\gamma$c gene and the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene were synthesized with a Model 394 DNA/RNA synthesizer (Applied Biosystems, Santa Clara, CA, USA) at the Central Research Laboratory of Okayama University Medical School. The sequences of these primers are shown in Table 1.

***Cells.*** A human monoblastic leukemia cell line, THP-1 and HTLV-1 infected T-cell line, MT-2 were maintained in culture in RPMI-1640 medium supplemented with 10 % fetal calf serum (FCS). For RNA analysis, the cells were cultured in 6-well culture plates at $1 \times 10^6$ cells/ml with or without stimulation. In some experiments, the viability of cells were checked with Trypan blue dye exclusion.

***RNA extraction, RT-PCR and semi-quantification of RT-PCR products.*** After the culture, the cells were harvested and washed with phosphate-buffered saline. Total RNA was extracted with RNAzol B (Biotecx, Houston, TX, USA). The amount of the extracted RNA was measured by absorbance at 260 nm. cDNA was synthesized from 3$\mu$g of total RNA with a Superscript preamplification system (Gibco BRL, Gaithersburg, MD, USA) according to the manufacturer's recommendations. cDNA derived from 100ng of total RNA was added to 23$\mu$l of a reaction mixture containing 0.5 units of Taq DNA polymerase (Takara, Otsu, Japan), 1 X PCR reaction buffer (Takara), 0.2 mM dNTPs and 1$\mu$M specific primers. RT-PCR and semiquantification of RT-PCR products were performed as described previously (25). Briefly, cDNA samples were subjected to 26 cycles of PCR for the $\gamma$c chain cDNA, 30 cycles for the other cytokine receptor cDNAs, and 18 cycles for GAPDH cDNA. Each PCR cycle consisted of 1min of denaturation at 94°C, 2min of primer annealing at 58°C, and 2min of extension at 72°C. Under these conditions, exponential amplification of specific cDNA was observed (data not shown). PCR products were separated by electrophoresis in 1.0% agarose gel containing 0.5mg/ml of ethidium bromide. The gels were UV-transilluminated and photographed with Polaroid 665 films (Polaroid, Cambridge, MA, USA). The negative films were developed for 1min at 20 °C. The films were scanned with an image scanner IX-4015 (Canon, Tokyo, Japan), and the densities of the bands were quantitated with NIH Image version 1.57 using an Apple Macintosh computer. Levels of the $\gamma$c chain mRNA were normalized by the level of GAPDH mRNA.

Table I Sequence of PCR primers

| | | |
|---|---|---|
| GAPDH | Foward | GGT GAA GGT CGG AGT CAA CGG A |
| | Reverse | GAG GGA TCT CGC TCC TGG AAG A |
| IL-2R$\alpha$ | Forward | GAC AGA AAT GGC TGC AAC CAT G |
| | Reverse | GAA CTG GGA AGT TGG AAT GAG ATG |
| IL-2R$\beta$ | Forward | TCC AAG AAC TCC AGG GTC AG |
| | Reverse | GCA AGG TTT TGA ACC GAG G |
| IL-2R$\gamma$ | Forward | CCA GGA CCC ACG GGA ACC CA |
| | Reverse | GGT GGG AAT TCG GGG CAT CG |
| IL-4R | Forward | GAA ATG TCC TCC AGC ATG GG |
| | Reverse | GGG TCT GGC TTG AGC TCT GAG C |
| IL-7R | Forward | GGA CTG CCA GAT TCA TAG GGT G |
| | Reverse | TTG TCG CTC ACG GTA AGT TCA G |

PCR: polymerase chain reaction; IL: interleukin.

## Results

***Expression of the $\gamma$c chain and associated cytokine receptor mRNA in THP-1 cells.*** RNA was extracted from resting THP-1 cells and subjected to RT-PCR for the $\gamma$c chain and the associated

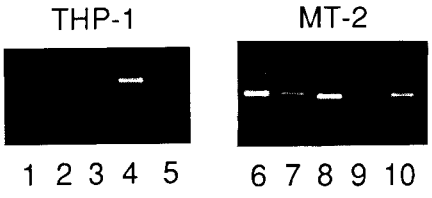


Fig. 1 Expression of common $\gamma$ chain ($\gamma$c chain) and associated cytokine receptors in THP-1 cells. Tatal RNA of THP-1 and MT-2 were subjected to RT-PCR analysis for mRNAs of interleukin 2 receptor $\alpha$ chain (IL-2R$\alpha$) (lanes 1 and 6); IL-2R$\beta$ chain (lanes 2 and 7) and IL-2R $\gamma$ chain (lanes 3 and 8); IL-4R (lanes 4 and 9) and IL-7R (lanes 5 and 10).

cytokine receptor mRNAs (Fig. 1). In the unstimulated THP-1 cells, the γc chain and IL-4R mRNAs were expressed. IL-2Rα, IL-2Rβ, and IL-7R were not expressed in THP-1 cells.

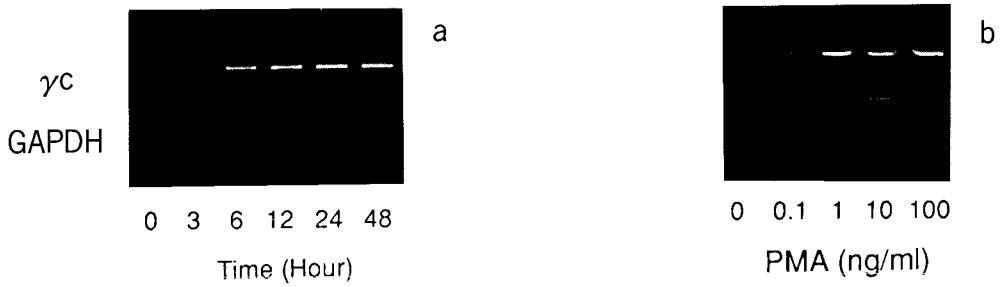


**Fig. 2** Effect of phorbol myristate acetate (PMA) on common γ chain (γc chain) mRNA expression in THP-1 cells. Time course of γc chain mRNA expression in PMA (10ng/ml)-treated THP-1 cells (**a**) and dose effect of PMA in 12-h cultures (**b**). Photographs show one representative result of three independent experiments.

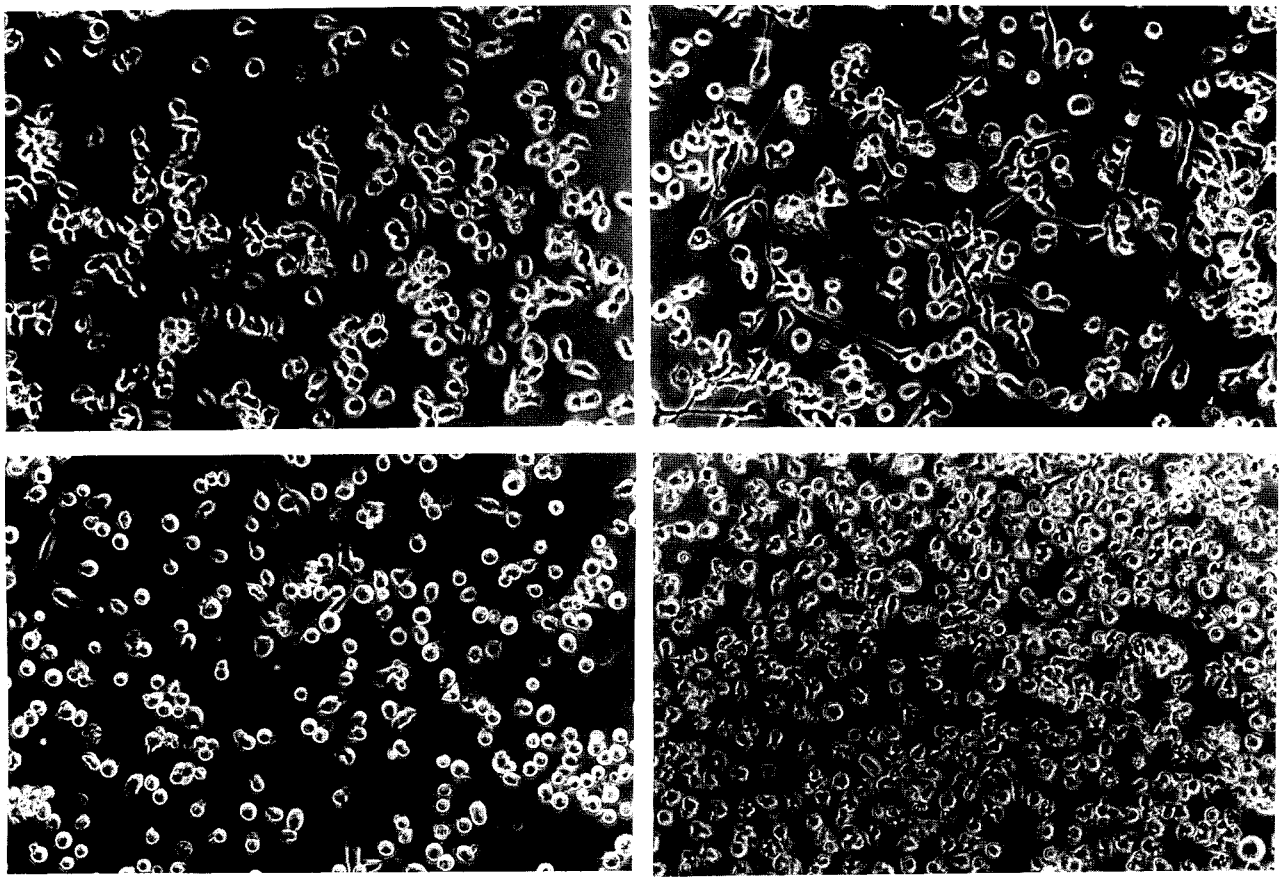

**Fig. 3** Effect of protein kinase inhibitors on PMA-induced morphological changes in THP-1 cells. Cells were cultured for 12h with medium containing 0.1 % DMSO (**a**), 10ng/ml of PMA (**b**), PMA + H-7 (20μM) (**c**), and PMA + staurosporine (50nM) (**d**). PMA-treated THP-1 cells adhered to flasks, spreaded, and formed processes. Although H-7 had a mild effect, staurosporine completely inhibited the cell adhesion and the morphological changes induced by PMA. PMA: See the legend to Fig. 2.

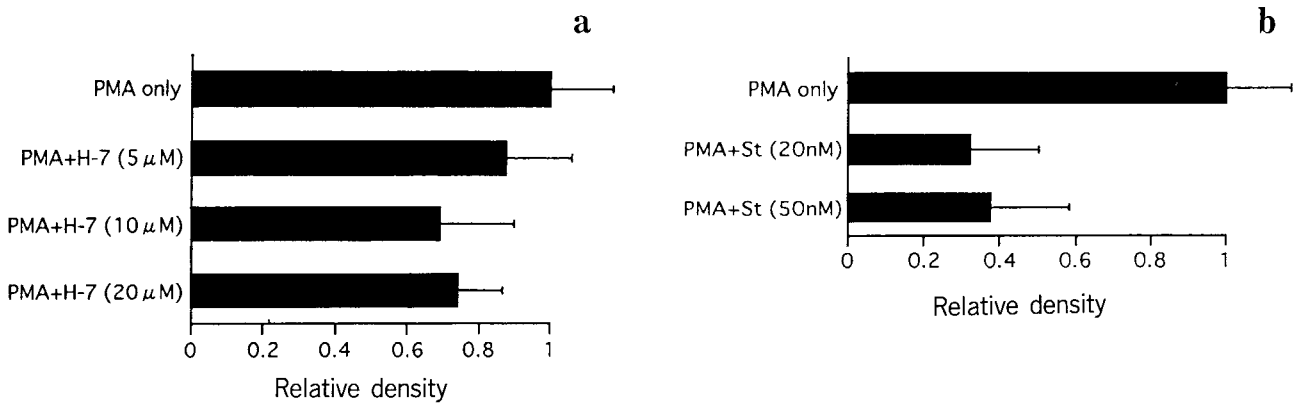


**Fig. 4** Effect of protein kinase inhibitors on γc chain mRNA expression in THP-1 cells. Cells were treated with PMA (10ng/ml) and H-7 (**a**) or staurosporine (**b**) at the concentrations indicated for 12h. The γc chain mRNA expression was analyzed by semiquantitative RT-PCR as described in Materials and Methods. Data are normalized by setting the relative γc chain cDNA density of PMA-treated THP-1 cells to 1.0. Data are presented as the mean ± S.D. of three independent experiments. St: Staurosporine. PMA and γc chain: See the legend to Figs 1, 2.

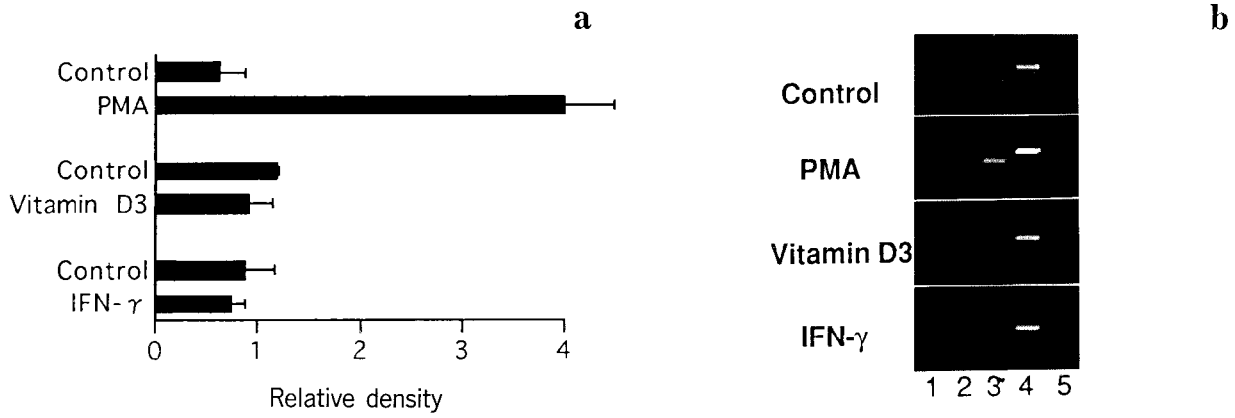


**Fig. 5** Effect of differentiation inducers on mRNA expression of the γc chain and of associated-interleukin (IL) receptor subunits in THP-1 cells. THP-1 cells were treated with medium containing 0.1% DMSO (control), PMA (10ng/ml, 12h), 1α, 25(OH)$_2$D$_3$ ($10^{-7}$M, 48h), and interferon-γ (IFN-γ) (500U/ml, 48h). Semiquantitative RT-PCR analysis was performed as described in Materials and Methods. **a**. Densitometric analysis of the γc chain cDNA. Data are presented as the mean density ± S.D. of three independent experiments. **b**. Photographs showing the representative result of RT-PCR. Lane 1, IL-2Rα; lane 2, IL-2Rβ; lane 3, γc chain; lane 4, IL-4R; lane 5, IL-7R. PMA and γc chain: See the legend to Figs 1, 2.

***Regulation of γc chain mRNA expression by PMA and protein kinase inhibitors in THP-1 cells.*** As Fig. 2 shows, THP-1 cells constitutively expressed γc chain mRNA only at a low level; however, the level began to increase 3h after PMA treatment and reached a plateau at around 12h. The maximal effect was obtained at 1ng/ml PMA. PMA treatment also induced macrophage-like differentiation in THP-1 cells which showed morphological changes, adhered to the plastic flasks (Fig. 3b), and expressed CD11b mRNA, a differentiation marker of monocyte-macrophage lineage (data not shown). To examine the effects of protein kinase inhibitors, THP-1 cells were cultured with 10ng/ml of PMA and various concentrations of H-7 or staurosporine for 12h. The expression of γc chain mRNA in PMA-treated THP-1 cells was reduced to about 70% by the addition of 20μM of H-7 (Fig. 4); this amount of H-7 inhibited cell adhesion slightly and was not cytotoxic at the duration used in this study (Fig. 3c). The effect of a higher concentration of H-7 was not examined because of cytotoxicity to THP-1 cells. Twenty nanomolar staurosporine was sufficient to

inhibit the morphological changes and cell adhesion induced by PMA (Fig. 3d) and suppressed the PMA-induced increase in γc chain mRNA expression to about 40% (Fig. 4). Under these conditions, staurosporine was not cytotoxic to THP-1 cells. In these experiments, H-7 and staurosporine did not affect the expression of GAPDH mRNA (data not shown). Expression of IL-2Rα, IL-2Rβ, IL-4R, and IL-7R was not affected by PMA treatment (Fig. 5).

***Effect of 1α, 25(OH)$_2$D$_3$ and IFN-γ on γc chain mRNA expression in THP-1 cells.*** To examine the effect of other differentiation inducers, 1α, 25(OH)$_2$D$_3$ and IFN-γ, on γc chain mRNA expression, THP-1 cells were treated with $10^{-7}$ M of 1α, 25(OH)$_2$D$_3$ or 500 U/ml of IFN-γ for 48h. Under these culture conditions, CD14 and HLA-DR mRNAs were induced by 1α, 25(OH)$_2$D$_3$ and IFN-γ, respectively (data not shown). 1α, 25(OH)$_2$D$_3$ had no effect on the γc chain mRNA level in THP-1 cells (Fig. 5). These reagents had no effects on the other γc chain-associated cytokine receptors (Fig. 5).

## Discussion

In the present study, we analyzed the γc chain gene regulation in the course of macrophage-like differentiation and signal transduction concerned with the γc chain gene expression in a monoblastic cell line, THP-1. Previous reports (14, 15) described that the γc chain mRNA was not detected in unstimulated THP-1 cells by Northern blot analysis; however, we performed RT-PCR to detect the γc chain mRNA and a low level of this mRNA was found in THP-1 cells. This discrepancy may be due to the sensitivity of the methods used. In the U-937 and HL-60 myelomonocytic cell lines, a significantly larger amount of γc chain mRNA was expressed as reported previously (data not shown) (13, 15). The differences in the expression of γc chain mRNA among these myelomonocytic cell lines may be explained by their different stage of maturation in monocyte-macrophage differentiation (18), or this may simply reflect the heterogeneity of monocytes. For example, a recent flow cytometric analysis revealed the presence of a γc chain-negative subpopulation among CD14$^+$ monocytes (12).

Myelomonocytic cell lines can be induced to differentiate into monocyte macrophage-like cells by treatment with PMA, 1α, 25(OH)$_2$D$_3$, or IFN-γ (19). These reagents act via different pathways and induce a different type of monocyte-macrophage differentiation on THP-1 (17, 20). The present results showed that, among these different inducers of monocyte-macrophage differentiation, only PMA up-regulated the γc chain mRNA expression. Whether this up-regulation induced by PMA is associated with differentiation or only a coincidental event remains unclear. Interestingly, although PMA treatment remarkably up-regulated the γc chain mRNA expression, PMA did not affect the expression of the other examined γc chain-associated interleukin receptor subunit mRNAs in THP-1 cells. These findings suggest that different regulatory pathways exist in these receptor genes.

Since PMA is known as an activator of protein kinase C (PKC), we examined the effects of protein kinase inhibitors on PMA-induced γc chain mRNA up-regulation. In the present study, H-7 reduced slightly the γc chain mRNA expression and staurosporine significantly suppressed the PMA-induced increase in γc chain mRNA expression. These results suggest that the PKC-dependent pathway may participate in the regulation of γc chain mRNA expression. Since the inhibitory effects of H-7 and staurosporine are not PKC-specific (21), however, the possibility that PKC-independent pathways also affect the regulation of γc chain mRNA expression can not be denied.

The present data showed that THP-1 cells constitutively express IL-4R mRNA. It has been reported that IL-4 has various effects on THP-1 cells (22, 23). Because γc molecules are not detected in unstimulated THP-1 cells, the effects of IL-4 may be mediated by a γc chain-independent pathway. Watanabe *et al.* also described that classical IL-4R (IL-4Rα) and γc chain transduce different signals in murine B cells (24). The present results suggest that formation of the IL-4Rα-γc complex is induced on THP-1 cells by PMA treatment. Alterations of IL-4R complex and the response to IL-4 of THP-1 cells are subjects for future study.

Although PMA is an artificial inducer of monocyte-macrophage differentiation for THP-1 and other myelomonocytic cell lines, PMA treatment of THP-1 cells can serve as an experimental model of γc chain mRNA up-regulation associated with monocyte-macrophage differentiation.

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