VJ REARRANGEMENT AT PREVIOUSLY EXCLUDED Igλ ALLELE IN HUMAN PLASMA CELL LINE NAT-30

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NAT-30 (μ,λ) is a human plasma cell line derived from Namalwa, a human Burkitt lymphoma cell line (1). It was previously demonstrated that concanavalin A (Con A) stimulation of the NAT-30 cells caused the loss of the original light chain of the NAT-30 cells and the expression of the new light chains in this cell line (2). This phenomenon was referred to as Light Chain Shifting (2). In this report, we showed that the new VJ rearrangement followed by the transcription spontaneously occurred in NAT-30 cells without Con A stimulation at low frequency and Con A stimulation may enhance this phenomenon in the magnitude of several hundreds fold.

Abstract

NAT-30 (μ,λ) is a human plasma cell line derived from Namalwa, a human Burkitt lymphoma cell line (1). It was previously demonstrated that concanavalin A (Con A) stimulation of the NAT-30 cells caused the loss of the original light chain of the NAT-30 cells and the expression of the new light chains in this cell line (2). This phenomenon was referred to as Light Chain Shifting (2). In this report, we showed that the new VJ rearrangement followed by the transcription spontaneously occurred in NAT-30 cells without Con A stimulation at low frequency and Con A stimulation may enhance this phenomenon in the magnitude of several hundreds fold.

Introduction

The Light Chain Shifting is a curious phenomenon that the human plasma cell line NAT-30 (μ,λ) changes its light chain to another one (2). Culture with concanavalin A (Con A) for one month, more than 10% of the NAT-30 cells carried out Light Chain Shifting (2). It was already shown that these new light chains were derived from another VJ rearrangement occurred at the previously excluded allele of the λ light chain locus (2). It has been considered that VJ rearrangement event is strictly regulated and never happens in plasma cell stage (3,4). The Light Chain Shifting shows the potential for the occurrence of another VJ rearrangement in plasma cells. Investigating this phenomenon will be profitable for understanding of the regulation mechanism of VJ rearrangement and allelic exclusion in human B lineage cells, which are not understood well.

In this paper, we investigated whether Light Chain Shifting occurs or not in the unstimulated NAT-30 cells by detecting mRNA and VJ coding joints for new light chains in the cDNA and genomic DNA of unstimulated NAT-30 cells.
Results

Detection of mRNA for new light chains in the unstimulated NAT-30 cells

Some of the new light chains, derived by Light Chain Shifting, were already cloned. We have tested whether two of these new light chains, $\lambda$CA2 and $\lambda$CA3 (Fig. 1) were expressed in the unstimulated NAT-30 cells or not using RT-PCR. Total RNA from unstimulated NAT-30 cells were extracted using Trizol reagent (GIBCO, BRL, UK) and served for cDNA synthesis as described elsewhere (5). Sense direction primers specific for variable region of the $\lambda$CA2 chain (P-V$\lambda$CA2, 5'-ACT-CTg-AgC-AgT-ggg-CAC-Ag-3'), $\lambda$CA3 chain (P-V$\lambda$CA3, 5'-AgT-ggC-AgC-ATT-gGC-AgC-AAC-3') and original $\lambda$ chain $\lambda$C5 (P-V$\lambda$C5, 5'-AAC-AgC-TCC-AAC-ATT-ggg-Ag-3') and anti-sense direction primer specific for original $\lambda$ chain constant region (P-Cl, 5'-gAA-gCT-CCT-CCA-gAg-ggg-gg-3') were used for PCR amplification to detect the expression of the $\lambda$CA2, $\lambda$CA3 and original $\lambda$ chain $\lambda$C5 (Fig. 2). We could detect not only the expression of the original $\lambda$ chain but also the expression of the new light chains, suggesting the possibility that the Light Chain Shifting occurs spontaneously in unstimulated NAT-30 cells (Fig. 2). However, it should be noted that unstimulated NAT-30 cells does not express any $\lambda$ light chains as proteins except original $\lambda$ chain $\lambda$C5 (2).

Fig. 1 DNA sequences of light chain variable regions from original NAT-30 ($\lambda$C5), new light chain producers CA2 ($\lambda$CA2) and CA3 ($\lambda$CA3).