MOLECULAR CLONING AND CHARACTERIZATION OF LEUKOTRIENE B\textsubscript{4} RECEPTOR

Takashi Izumi, Takehiko Yokomizo, Toshio Igarashi, and Takao Shimizu

The Department of Biochemistry and Molecular Biology, Faculty of Medicine, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113, Japan

INTRODUCTION

Leukotriene B\textsubscript{4} (LTB\textsubscript{4}) is a potent proinflammatory and chemotactic factor biosynthesized in various tissues, which is involved in inflammation, immune responses and host defense against infection.\textsuperscript{1} LTB\textsubscript{4} activates inflammatory cells by binding to its cell-surface receptor (BLT). Specific binding for \textsuperscript{3}H\textsubscript{4}LTB\textsubscript{4} was reported in the membrane fractions of granulocytes, T lymphocytes, alveolar macrophages, spleen, brain, THP-1 cells, and differentiated HL-60 cells.\textsuperscript{2-5} By addition of GTP\textsubscript{y}S, low affinity sites of \textsuperscript{3}H\textsubscript{4}LTB\textsubscript{4} binding appeared in spleen membranes.\textsuperscript{5} LTB\textsubscript{4} stimulated a dose-dependent increase in GTP hydrolysis in HL-60 cells.\textsuperscript{6} LTB\textsubscript{4} induced increase in intracellular calcium, D-myo-inositol-1,4,5-triphosphate (InsP\textsubscript{3}) accumulation, O\textsubscript{2}\textsuperscript{-} production, and activation of GTPase in a pertussis toxin (PTX)-sensitive manner.\textsuperscript{7,8} On the other hand, LTB\textsubscript{4}-induced hyperadhesiveness of vascular endothelial cells was reported to be PTX-insensitive.\textsuperscript{9} These data indicated the presence of BLT coupled with G-protein(s). However, BLT had not been purified to homogeneity or cDNA-clone.

We cloned a cDNA for BLT from HL-60 cells differentiated with retinoic acid using a subtraction strategy.\textsuperscript{10} The open reading frame (ORF) of the cDNA encodes a protein of 352 amino acids and is predicted to contain seven membrane-spanning domains. Membrane fractions of Cos-7 cells transiently transfected with an expression construct containing the ORF of the BLT showed specific binding of LTB\textsubscript{4} with a K\textsubscript{d} of
0.154 nM. In CHO cells stably expressing BLT, LTB4 elicited many signal transductions such as increase in intracellular calcium, InsP3 accumulation, and inhibition of adenylyl cyclase. The CHO cells revealed a marked chemotactic response toward nM order of LTB4. Calcium increase and InsP3 accumulation induced by LTB4 were partially sensitive to pertussis toxin (PTX), while chemotaxis and inhibition of adenylyl cyclase were completely sensitive to PTX, indicating that BLT transduces LTB4 signals through both PTX-sensitive and -insensitive G proteins.

**MOLECULAR CLONING OF LTB4 RECEPTOR (BLT)**

When HL-60 human leukemia cells were treated with 1 μM retinoic acid, they were differentiated into granulocyte-like cells with an increase of [3H]LTB4 binding. The binding was maximal after 4 days of differentiation. Two cDNA pools were synthesized from poly(A)+ RNAs from undifferentiated and retinoic acid-differentiated HL-60 cells. A subtracted cDNA was obtained using a polymerase chain reaction (PCR)-Select cDNA subtraction kit (Clontech), subcloned into a T-vector (Promega), and sequenced. Among 66 clones, one clone was found to encode the 3'-UTR of a seven-span orphan receptor previously reported. Two cDNAs (named HL-1 and HL-5) containing the corresponding ORF of this orphan receptor were isolated from a day-3 differentiated HL-60 cDNA library. HL-1 and HL-5 had different 3'-UTR but the same ORF.