G101 Cloning of transcriptional control regions of TNF-alpha

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TNF-alpha(tumor nacrosis factor) is an important mediator of inflammatory and immune functions. TNF-alpha and TNF-beta(lymphotoxin) are closely linked on mouse chromosome 17. In order to investigate interactive effects of transcriptional control regions of the TNF genes [TNF-alpha promoter, TNF-beta promoter and 3′ -untranslated regions(3′ UTR)] in response to exogenous signals in TNF-alpha gene expression, we initially performed PCR. PCR products of TNF-alpha promoter, TNF-beta promoter and 3′ UTR were respectively 1.92, 1.84 and 1 kilobases in size and were cloned into the pBluescripts or pUC 19. Their nucleotide sequences were identified at both side ends and middle position by sequencing analysis. We devised two luciferase reporter constructs respectively containing TNF-alpha promoter and both TNF-alpha promoter and 3′ UTR. The luciferase reporter constructs is being transiently transfected into a macrophage cell line, J774A, by electroporation. (HRC-95-0305)

G102 Antisense Oligonucleotides against $p56^{lck}$ Block the Differentiation of Immature $CD4^+CD8^+$ Thymocytes to Mature $CD4^+$ or $CD8^+$ Thymocytes

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T cell precursors from bone marrow migrate to thymus and differentiate along a pathway that generates distinct T cell subpopulations. The earliest thymocytes lacking detectable CD4 and CD8 (double negative thymocytes:DN) develop through an intermediate stage expressing both CD4 and CD8 (double positive:DP) to a mature $CD4^+$ or $CD8^+$ (single positive:SP) thymocytes. These developmental processes, including positive and negative selections, are very complex and seem to involve signal transduction events. Results from CD45(protein tyrosine phosphatase)-deficient mice suggest a possibility that a signaling process is required to generate mature SP thymocytes from DP intermediates. To test whether a protein tyrosine kinase (PTK), especially $p56^{lck}$, is required for immature DP thymocytes to become mature SP cells, the processes of thymocyte development were followed using fetal thymic organ culture (FTOC) technique. When thymocytes were treated with PTK inhibitors at their initial DN stage, only a few DP and SP thymocytes were produced and the number of total thymocytes were much less than that found in $p56^{lck}$-deficient mice. These data suggests that PTK(s) other than $p56^{lck}$ is involved in early stage of development. PTK inhibitors were also treated when most thymocytes were at DP stage. After 4-5 days of culture, production of mature SP thymocytes were greatly reduced and most DP thymocytes disappeared. When we treated the culture with antisense oligonucleotide against $p56^{lck}$ at the same stage of FTOC, the development of DP to SP thymocytes was blocked. These results suggest that $p56^{lck}$ is required for the development of intermediate DP thymocytes into a fully mature SP thymocytes.