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Antigen Receptor-Mediated Induction of Cytolytic T cell-Specific Transcripts Expression

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ABSTRACT

Employing the approach to isolate the genes expressed preferentially in cytolytic T cell (CTL) but not in other types of cell, 3 CTL-specific cDNAs were recently cloned. To characterize these cDNA clones in relation to CTL activation, their expression pattern after T cell antigen receptor (TCR) or interleukin 2 (IL-2) stimulation were investigated by RNA blot analysis of cloned CTL L3 cells. Transcripts level of two cDNA clones were markedly elevated by TCR stimulation but not by IL-2. In addition, transcripts expression of both clones were abrogated by cyclosporin A treatment. These results indicated that gene activation mediated by TCR is distinct from that mediated by IL-2 and imply that those two unidentified cDNA clones are related to TCR-mediated, IL-2-independent but cyclosporin A-sensitive pathway for CTL activation.

Key Words: CTL activation, T cell antigen receptor, IL-2, cyclosporin A

INTRODUCTION

T cells can be activated by specific antigen, monoclonal antibody (mAb) directed against T cell antigen receptor (TCR) or mitogenic lectins to express the new surface molecules including interleukin 2 (IL-2) receptor, to produce lymphokines and eventually to proliferate (Moldwin *et al,* 1986; Helmer *et al,* 1984; Herold *et al,* 1984; Cantrell and Smith, 1983). According to current concept of T cell activation, proliferation of both conventional cytolytic T cell (CTL) and helper T cell (Th) is IL-2-dependent. But several CTL clones which do not secrete IL-2, can proliferate by specific antigen in absence of exogenous IL-2 (Kelso and Glasebrook, 1984). Moreover, it has been shown that even conventional CTL's can proliferate by TCR stimulation without exogenous IL-2 (Moldwin *et al,* 1986). These findings suggest that TCR-driven, IL-2-independent CTL activation may involve different biochemical pathways from that used in Th and may be associated with the expression of CTL-specific genes. In this regard, identification and characterization of T cell-specific or subset-specific genes provide important information to understand T cell activation mechanism. Recently we have cloned several T cell- or subset-specific cDNAs from Th and CTL (Kwon *et al,* 1987). To characterize these cDNA clones further in relation to CTL activation, cloned CTL L3 cells were stimulated through TCR or IL-2 and treated with cyclosporin A (CSA) because immunosuppressant CSA can inhibit the certain components involved in T cell activation (Orosz *et al,* 1982, 1983). In this paper we describe the existence of TCR-driven, IL-2-independent and CSA-sensitive pathway of CTL gene activation.

MATERIALS AND METHODS

Cells

Cloned CTL L3 cells were maintained as previously described (Glasebrook and Fitch, 1980). L3 cells were stimulated with clonotypic mAb 384.5 directed against TCR (Lancki *et al,* 1983) or with recombinant human IL-2 (100 u/ml, Cetus Corp. Emeryville, CA). For the treatment of L3 with CSA,

cells were incubated with concanavalin A (Con A, 2μg/ml), Con A plus CSA (0.2 μg/ml) or Con A plus actionmycin D (1 μg/ml) for 6h at the cell concentration of 2.5×10^6/ml. IL-2-producing mouse T cell lymphoma EL4 (Farrar *et al,* 1980) and B cell lymphoma K46 (Kim *et al,* 1979) were maintained in RPMI medium containing 5% fetal calf serum.

RNA Blot Hybridization

Total cytoplasmic RNA (10 μg) or poly $(A)^+$ mRNA (1 μg) was fractionated on 1.2% agarose-formaldehyde gel (Thomas, 1980) and transferred to Gene Screen Plus (NEN, Boston, MA). Gel-purified inserts of 3 CTL-specific cDNAs (Kwon *et al,* 1987) were ^{32}P- labeled by nick translation and used as a probe. Filters were prehybridized at 42°C for 2h in 50% formamide, 5x SSC (1x SSC: 150mM NaCl, 15mM sodium citrate), 0.1% SDS, 250 μg/ml of salmon sperm DNA and 10% dextran sulfate. Hybridization was carried out in the same buffer with nick translated cDNA insert. Filters were washed at room temperature for 15 min in 2x SSC, 0.1% SDS and then at 42°C for 30 min in 0.1x SSC, 0.1% SDS with several changes.

RESULTS

Transcripts Expression of CTL-specific cDNA in L3 cells after TCR or IL-2 Stimulation

Expression of transcripts corresponding to CTL-specific cDNA clone L3G10#6 was not elevated after stimulation by anti-TCR monoclonal antibody 384.5 or by IL-2 as shown in Fig. 1a. In contrast to this, L3G 14#2 and L3G25#4 transcripts were inducible by TCR but not by IL-2 stimulation. Their transcripts level were markedly increased 6h after TCR stimulation and maintained the elevated level at least until 24h and in case of L3G14#2, it hybridized to two different bands of 21S and 12S (Fig. 1b, c).

Effect of CSA on CTL-specific Transcripts Expression

Expression of 3 CTL-specific transcripts was not detected in B cell line K46 cells nor EL4 cells which have a Th-phenotype. L3G10#6 was expressed constitutively in L3 cells while L3G14#2 and L3G25#4 were inducible by Con A. Constitutive expression of L3G10#6 was detected in other CTLs, CTLL All and CTLL 15G (Data no shown). By the treatment of CSA, expression of transcripts corresponding to L3G14#2 and L3G25#4 was completely blocked while level of L3G10#6 transcripts was not altered. However, actinomycin D abolish the expression of 3 CTL-specific transcripts completely (Fig. 2).

DISCUSSION

For this study, we chose three CTL-specific cDNA clones designated as L3G10#6, L3G14#2 and L3G25#4. As described previously (Kwon *et al* , 1987), the nucleotide sequence of L3G10#6 was

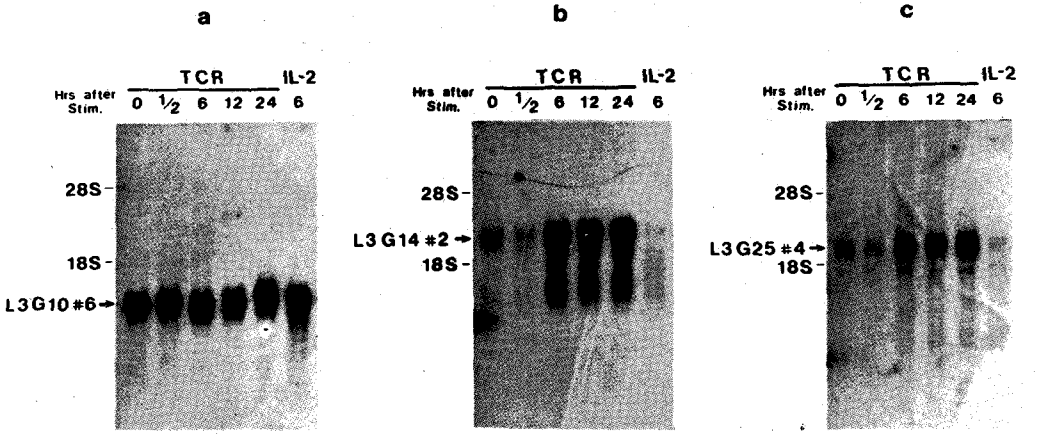

Fig. 1. Expression of CTL-specific transcripts in L3 cells after TCR-or IL-2 stimulation. L3 cells were stimulated with immobilized clonotypic mAb 384.5 directed against TCR for 0,1/2, 6, 12 and 24 h or 100 u/ml IL-2 for 6 h. Poly $(A)^+$ mRNA was fractionated, transferred to membrane and hybridized to the ^{32}P-labeled insert of CTL-specific cDNA.

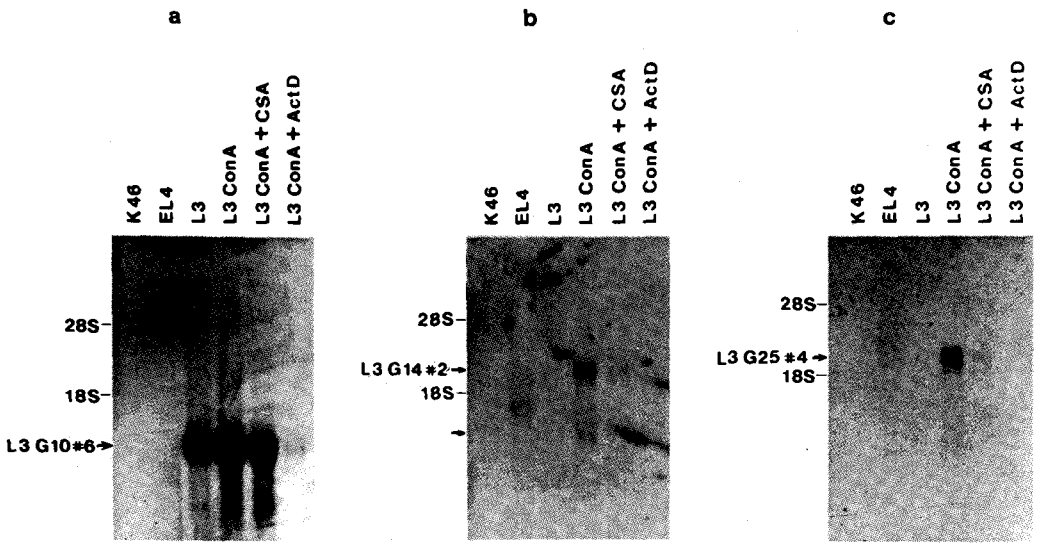

Fig. 2. Effect of cyclosporin A on the CTL-specific transcripts expression. L3 cell were stimulated with Con A (2μg/ml), Con A plus CSA (0.2μg/ml) or Con A plus actinomycin D (1 μg/ml). Total cytoplasmic RNA (L3) or poly (A)$^+$ mRNA (K46, EL4) was fractionated, transferred to membrane and hybridized to ^{32}P-labeled insert of CTL-specific cDNA.

homologous to CTL-specific serine esterase gene (Gershenfeld and Weissman, 1986) while sequence homologuous to L3G14#2 and L3G25#4 can not be identified. To determine whether the CTL-specific cDNAs are involved in CTL activation, cloned CTL L3 cells were stimulated through TCR with clonotypic mAb 384.5 up to 24h or with IL-2 for 6h. After stimulation, expression of transcripts corresponding to CTL-specific cDNAs was analyzed by RNA blot hybridization. As shown in Fig. la, transcripts level of L3G10#6 was not affected either by TCR or IL-2 stimulation. CTL-specific serine esterase has been implicated in cell-mediated cytolysis (Gershenfeld and Weissman, 1986; Lobe *et al,* 1986; Pasternack *et al,* 1986). Providing such an implication were correct, constitutive expression of L3G10#6 at high level and no alternation of transcripts level after TCR-stimulation may reflect the fact that cloned CTLs are continuously cytolytic, so cannot truely return to the non-activated state. When the RNA blot was probed with L3G14#2, two bands of different size were detected (Fig. 1b). The relation between these two mRNA has not been determined. They may arise from the same gene by differential splicing or alternative exon usage. In contrast to L3G10#6, expression of transcripts corresponding to L3G14#2 and L3G25#4 was markedly elevated by TCR stimulation but not by IL-2 (Fig. 1b and c). These results indicated that pathway for gene activation mediated by TCR-stimulation is distinct from that mediated by IL-2 and raised the possiblity that these cDNA clones may be related to antigen-driven, IL-2-independent pathway for proliferation of CTLs described recently (Moldwin *et al,* 1986). To confirm this possibility, we next examined the effect of CSA on their transcripts' expression. According to earlier studies (Orosz *et al,* 1982, 1983), CSA can suppress clonal proliferation of Th and CTL in a specific manner. CSA inhibited the antigen-driven component of proliferation of T cell clones without occluding TCR while exerted no or little effect on the IL-2-driven component. For this experiment, we stimulated L3 cells with Con A, instead of mAb 384.5, in presence of CSA because i) expression of L3G14#2 and L3G25 #4 was inducible by Con A as well as TCR stimulation, ii) although Con A was used instead of specific antigen to bypass the antigen-TCR interaction, the suppressive action of CSA was still effective (Orosz *et al* , 1983). As shown in Fig. 2, CSA treatment had no effect on the transcripts level of L3G10#6 whereas actinomycin D abolished its expression completely. On the contrary to this, accumulation of L3G14#2 and L3G25#4 transcripts in reponse to Con A were almost completely abrogated by CSA. These findings showed that suppressant action of CSA was a selective one unlike nonselective actinomycin D and was

in agreement with the previous results (Orosz *et al,* 1982; Herold *et al,* 1986) which demonstrated that TCR-mediated component was affected by CSA. However, recent study (Herold *et al,* 1986) described that CSA had no effect on TCR-mediated proliferation of L3 cells. Thus, these cDNA clones cannot be considered to be involved in antigen-driven, IL-2-independent CTL proliferation even with the facts that their expression was markedly induced by TCR-stimulation, not by IL-2. Previously it has been described that by CSA, lymphokine production of CTL was inhibited (Herold *et al,* 1986) and proliferation and lymphokine gene expression required distinct signals (Heckford *et al,* 1986). These studies leave the other possible relation of L3G14#2 and L3G25#4 to lymphokine production during CTL activation. And this possibility can be persued employing antisense RNA technology (Izant and Weintraub, 1984). Taken together, these cDNA clones may be useful to dissect the pathway of gene activation after Con A or TCR stimulation though exact biological function of these transcripts are not identified at present.

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=국문초록=

항원수용체자극에 의한 Cytolytic T cell 특이전사체 표현유도

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Cytolytic T cell(CTL)에서는 표현되나 다른 세포에서는 표현되지 않는 유전자를 검색하여 최근 저자는 3종의 CTL 특이cDNA를 cloning하였다.

CTL특이cDNA의 기능을 규명하기 위하여 CTL L3 cell을 항원수용체를 통하여 혹은 interleukin 2(IL-2)로 자극하여 활성화시킨 후 RNA blot analysis로 각 cDNA clone의 상응전사체 표현양상을 관찰, CTL활성화과정과의 연관성을 실험하였다.

이중 2종의 cDNA 상응전사체표현은 항원수용체자극에 의해 현저히 증가된 반면 IL-2는 전혀 영향을 미치지 않았으며 이 같은 전사체표현증가는 cyclosporin A 처리로 완전히 억제되었다.

이상의 결과는 항원수용체자극으로 활성화되는 유전자가 IL-2에 의해 활성화되는 유전자와는 상이함을 보여주는 것이며 또한 2종의 cDNA clone이 IL-2에 의해 활성화되지 않으나 항원수용체를 통하여 중개되며 cyclosporin A에 예민하게 반응하는 CTL활성화과정의 특정경로에 관여하는 것으로 사료된다.