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Comparison of gene expression profiles in mouse primary T cells under normal and prolonged activation

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Abstract

In order to investigate the global transcriptional change of mouse primary T cells after prolonged activation, we took advantage of a Mouse Genome 430 2.0 Array to assess and compare the overall gene expression profiles of mouse T cells after activated with anti-CD3/CD28 for 18 or 48 h. The results demonstrated that most activation-related genes were preferentially up-regulated in mouse primary T cells after stimulated for 18 h; some apoptotic genes, however, were also found to be moderately up-regulated simultaneously. After the activation of T cells for 48 h, lots of apoptosis-related genes were dramatically up-regulated, followed by the augmentation of activation-induced cell death. In general, the number of differentially expressed genes in T cells after activation over 48 h declined almost in half as compared to that of 18 h. Both microarray and cytokine content analyses revealed that Th1 cytokines, rather than Th2 cytokines, were specifically up-regulated in activated mouse primary T cells. The present study also identified a number of genes that were dramatically up or down-regulated in T cells activated for 48 h for the first time, although the exact functions of these proteins are not known. Our studies provide detailed information on genes expression profiles of mouse primary T cells after normal (18 h) and prolonged activation (48 h); these data may accelerate the understanding of the T cell activation process and offer clues to the therapy of immune diseases.

Keywords: T cells activation; Microarray; Anti-CD3/CD28; Apoptosis; Activation-induced cell death

Introduction

T cells activation is a complicated and highly cooperative process and one of the most important events in immune response. Under the normal physiological situation, lymphocytes are resting. The ability of naive T cells to proliferate and obtain potential functions depends on the strength of signals received by the T cell receptor (TCR/CD3) and a series of co-stimulatory receptors—the most prominent of which is CD28 [1]. The concurrent ligation of these receptors triggers signals that lead to the proliferation and differentiation of T cells. Evidence has revealed that naive T cells response to T cells receptor ligation will not only lead to the differentiation and generation of effector cells, but also trigger immature thymocytes to apoptosis and mature T cells to activation-induced cell death (AICD) [2,3].

Apoptotic cell death is an essential process involved in the regulation of cellular homeostasis during development, differentiation and other pathophysiological conditions; its deregulation contributes to many diseases including autoimmunity, cancer, acquisition of drug resistance in tumors, stroke and progression of some degenerative diseases [4,5]. During the immune response, it is critical for T cells to downsize their numbers after activation and proliferation because the unchecked proliferating T cells enhance the risk of malignancy and autoimmune diseases. This process is also called activation-induced cell death (AICD) [6]. The members of the tumor necrosis factor/nerve growth factor (TNF/NGF) receptor superfamily are the best-studied cell surface protein receptors involved in AICD. This constantly expanding superfamily includes receptors for TNF, TNF-related apoptosis-inducing ligand (TRAIL), CD95/APO-1 ligand (Fas L) and lymphotoxins [7].

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Recently, the signal transduction pathways underlying T cells activation or apoptosis triggered by TCR/CD3 have been described; however, the switch that determines whether primary T cells undergo activation-induced proliferation or apoptosis is still poorly understood. By using microarray analysis, data concerning the gene expression profiles of activated human T cells were described [8,9]. However, those studies only focused on the early stage of human T cells activation (not exceeding 24 h) rather than the stages after prolonged activation. In fact, the phenotype as well as the gene transcriptional changes of mouse primary T cells after prolonged stimulation was a critical step to fully appreciate the process of T cells immune response. In the present study, we describe the utilization of a Mouse Genome 430 2.0 Array (Affymetrix) to assess the differences in gene expression profiles of mouse primary T cells before and after stimulation with anti-CD3/CD28 for 18 or 48 h. The results of this study might provide more molecular evidences for the understanding of T cells activation and its associated AICD.

Table 1
The differentially expressed genes in primary T cells after stimulation for 18 or 48 h (at least 2-fold)

Description	Up-regulation		Down-regulation	
	18 h	48 h	18 h	48 h
Apoptotic protein	61	51	66	13
Cancer	21	23	36	7
Cell cycle regulator	121	88	98	22
Chaperone	105	93	58	23
Immunity protein	37	48	97	29
Microtubular protein	2	5	14	3
Motor	5	5	12	2
Nucleic acid binding protein	419	44	386	76
Transport	260	259	258	79
RNA	10	6	4	0
Structural protein	671	719	782	204
Enzyme	927	853	986	184

Materials and methods

Materials

The antibodies against CD3, CD28, Thy1.2 (biotin conjugated), biotin (FITC conjugated), CD4 (PE conjugated) and CD69 (FITC conjugated) were purchased from BD Pharmingen (San Diego, CA). Antibody against mouse caspase 3 was obtained from Cell signaling Technology (Beverly, MA 01915); antibodies against c-Jun and actin were from Santa Cruz Biotechnology (CA, USA). Streptavidin MicroBeads, LS column, Midi MACS Separator were purchased from Miltenyi Biotec (Germany). Mouse Th1/Th2 cytokine CBA kit was from BD Biosciences. Trizol was from Invitrogen. CFSE and 7 AAD were from Sigma (St. Louis, MO).

Cell culture

Female wild-type C57BL/6 mice, 5–6 weeks of age, were purchased from SIPPR-BK Experimental Animal Center (Shanghai, China) and housed in a pathogen-free environment. Mouse primary T cells suspension were prepared aseptically from lymph nodes and diluted into a density of 1×10^7 cells/ml with fresh RPMI 1640 medium (Gibco, USA) supplemented

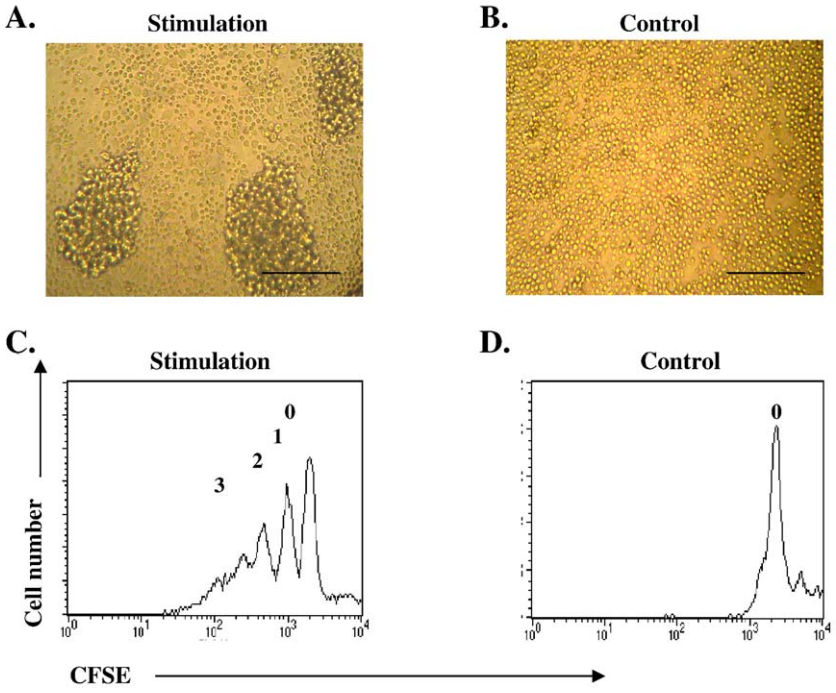

Fig. 1. Activation of primary T cells with anti-CD3/CD28. Primary lymphocytes were aseptically taken from mice lymph nodes, separated and diluted into 1×10^7 cells/ml, further cultured in RPMI 1640 for 18 h with (A) or without anti-CD3/CD28 (B). Cells were photographed with a KODAK camera (DX4330) (magnification 100×). Cells were pre-stained with CFSE for 10 min, then stimulated for 3 days with (C) or without (D) anti-CD3/CD28. PE–anti-CD4 was added before flow cytometric analysis. $CD4^+$ cells were gated for CFSE analysis. The number in panels C or D indicated the cell divisions.