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Molecular Immunology 42 (2005) 71–78
Molecular Immunology
www.elsevier.com/locate/molimm

Prominent dominant negative effect of a mutant Fas molecule lacking death domain on cell-mediated induction of apoptosis

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Received 2 June 2004; accepted 12 July 2004
Available online 11 September 2004

Abstract

Using a panel of transfectant B lymphoma cells expressing varying amounts of the mutant Fas together with the endogenous wild type Fas, semi-quantitative studies on the dominant negative effect of a murine mutant Fas molecule lacking death domain were carried out. In anti-Fas antibody-mediated induction of apoptosis, the mutant molecules exerted significant dominant-negative effect only when their expression level was comparable to or higher than that of wild type molecules, or when exposed to low amounts of the antibody. The inhibitory effect was accompanied by the failure in DISC formation in spite of Fas aggregation. When they were subjected to T cell-mediated Fas-based induction of apoptosis, however, the dominant negative effect was prominent such that the expression of even a small amount of the mutant molecules resulted in significant inhibition. Such a strong inhibitory effect explains the dominant phenotype of this type of mutant Fas molecules in ALPS heterozygous patients and also implies that the physiological effectors for Fas in vivo are cells, i.e., FasL-expressing activated T cells.

Keywords: Fas; ALPS; Death domain; Dominant negative effect; DISC

1. Introduction

The homeostasis of the cells of the immune system is maintained by programmed cell death as well as by cell proliferation. A number of genes are involved in the processes of programmed cell death. Among them, Fas (CD95, APO-1) is an apoptosis-inducing cell surface receptor belonging to the tumor necrosis factor receptor (TNFR) super family. A defect in the Fas gene is known to results in lymphoproliferative autoimmune disorders characterized by massive proliferation of $CD4^{-}8^{-}$ T cells and autoantibody production, as best exemplified by lpr mice (Watanabe-Fukunaga et al., 1992). Similar human diseases termed autoimmune lymphoproliferative syndrome (ALPS-1a) have also been identified as hereditary diseases caused by mutated Fas genes (Vaishnaw et al., 1999; Straus et al., 1999). Although the murine lpr gene is recessive, some of human ALPS genes have been found to be dominant. The common feature among such dominant negative ALPS genes has been shown to be a defective cytoplasmic death domain associated with an intact extracellular portion (Jackson et al., 1999). Functional studies on cells transfected with the genes with such a defect utilizing anti-Fas antibody-mediated cross-linking confirmed their dominant negative effect on wild type Fas genes (Cascino et al., 1996; Siegel et al., 2000). The Fas-mediated apoptotic signal is transduced through the formation of death-inducing signal complex (DISC) consisting of Fas, FADD and caspase 8 (Walczak and Sprick, 2001). It has been reported that the formation of Fas hexamer (paired trimer) is essential for DISC formation (Holler et al., 2003). It is speculated that mutant Fas molecules carrying a defective cytoplasmic death domain interfere with the transduction of the death signal of wild type molecules by forming defective trimers (Siegel et al., 2000).

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0161-5890/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.molimm.2004.07.042

The most likely physiological effectors of Fas mediated lysis are cells expressing Fas-ligand (FasL). Although soluble form of FasL is released into tissue fluid in certain inflammatory situations, such soluble molecules fail to induce death signal and can even exert an inhibitory effect (Suda et al., 1997; Schneider et al., 1998). Thus, it is essential to study the effect of the dominant negative mutant molecules on cell-mediated FasL-based induction of apoptosis. In this report, we have developed a defective murine Fas cDNA lacking death domain with the intact extracellular portion. Using this mutant Fas gene, we have carried out semi-quantitative analyses of its influence on the function of wild type Fas molecules on the death signal transduction in cell-mediated as well as in antibody-mediated Fas-based induction of apoptosis.

2. Materials and methods

2.1. Cells

BK1, a BALB/c-derived KLH-specific $CD4^+$ T cell clone with lytic activity (Takayama et al., 1991), and 4BO, an OVA-specific $CD4^+$ bulk T cell line established from a BALB/c mouse were established by us and were maintained in vitro with regular antigenic stimulations. These T cell lines had been shown to be capable of exclusively Fas-dependent antigen-specific cell lysis (Takayama et al., 1991; Hanabuchi et al., 1994). A20.2J was a BALB/c-derived class II positive B lymphoma (Chesnut et al., 1982) expressing endogenous Fas.

2.2. Cell culture

Cells were cultured in Dulbecco's Modified Essential Medium (DMEM: Invitrogen, Carlsbad, California) supplemented with 2 mM non-essential amino acids, 1 mM sodium pyruvate, 2 mM extra glutamine, 5×10^{-2} mM 2-mercaptoethanol (2-ME), penicillin, streptomycin (100 units/ml), 10 mM HEPES buffer (pH 7.2), and 10% pre-screened heat-inactivated fetal calf serum (FCS). For the maintenance of T cell lines, the medium was further supplemented with 5% of 48 h supernatant of Con A-stimulated LEW rat spleen cell culture which was passed through a Sephadex G-10 column to remove Con A.

2.3. Mutant Fas and transfection

The mutant Fas cDNA was constructed by PCR on murine Fas cDNA as a template using following two primers, one carrying a mutated sequence. Fas-del-N (sense orientation: 5′-GCGAATTCTGCAGACATGCTGTGGATCTGG-3′) consisted of N-terminal 23mer including the initiation codon and an upstream addition of an *Eco*RI cloning site. Fas-del-C (antisense orientation: 5′-GCGAATTCCTACTCAGCTGTGTCTTGGATGC-3′) carried a base substitution within the death domain as shown in Fig. 1 and also had a down stream

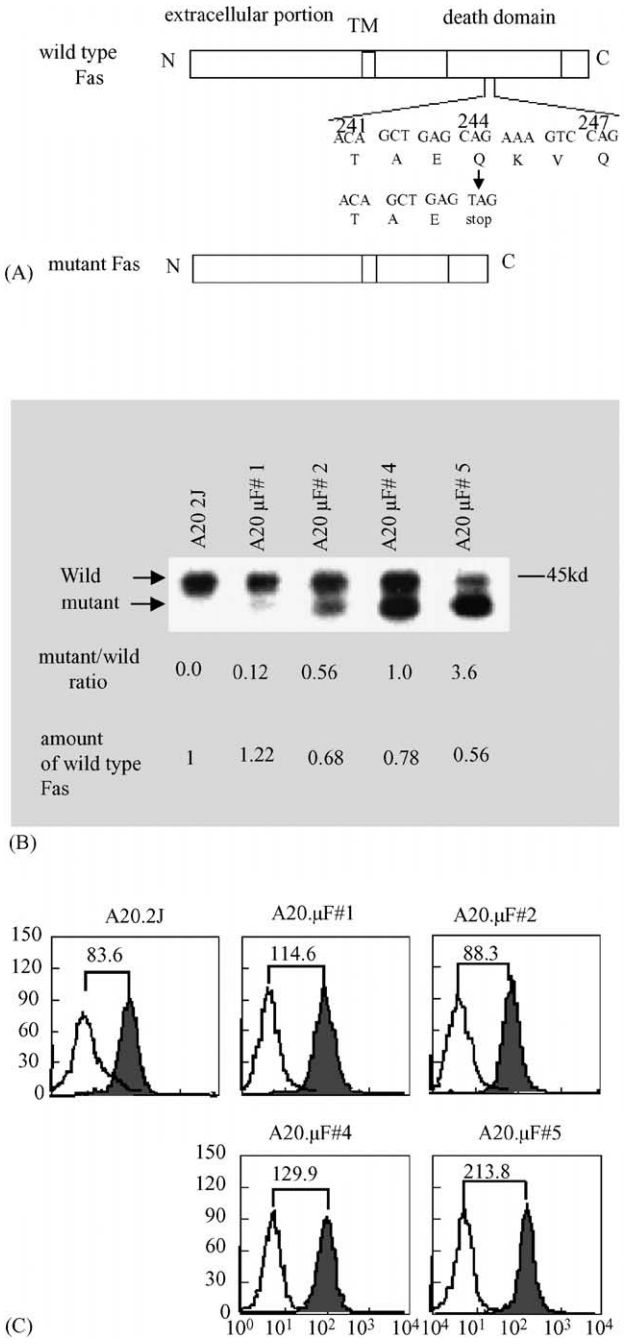

Fig. 1. Mutant Fas and transfectants. (A) The murine mutant Fas cDNA was constructed by introducing a base substitution within the death domain changing the 244th codon (Q) to a stop codon. (B) Surface labeled Fas molecules on transfectants clones were precipitated with anti-Fas antibody and analyzed by Western blotting using streptavidin-peroxidase. The wild type and mutant Fas molecules were distinguishable by molecular weight difference. Mutant/wild type ratio was calculated from the density of the two bands. The amount of wild type Fas indicates the relative amount (A20.2.J = 1) of wild type Fas expressed on the cell surface calculated from the mutant/wild type ratio and the total amount of surface-stained Fas shown in panel C. (C) Staining profiles of A20.2.J and transfectant clones. The cells were stained with biotin-anti-Fas antibody followed by streptavidin-FITC. Negative staining was performed by staining the cells with the secondary reagent alone. The amount of surface Fas (wild type + mutant) is defined as net voltage difference between the unstained and stained peaks.