A Novel Mutation in CD40 Ligand Gene in a Sporadic Patient with Hyper-IgM Syndrome

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Abstract
Hyper-IgM syndrome is a rare immunodeficiency disease characterized by low or absent IgG, IgA, and IgE levels but normal or elevated level of IgM. It can occur as an acquired form or X-linked or autosomal mode of inheritance. The X-linked form (HIGM1) is a result of mutations in the CD40L ligand (CD40L) gene. We investigate the expression of CD40L on activated T cells from a sporadic male case of HIM with no family history and the B cell function in response to anti-CD40L mAb and cytokines. Staining of CD40L on activated T cells from the patient is negative with recently developed anti-human CD40L mAb, M90 and M92. The low expression of CD40L on activated T cells from the patient’s mother is also detected. Sequencing of CD40L coding region reveals a 4 bp deletion within the CD40L binding domain. These results indicate that the patient has X-linked inheritance pattern (XIGM1). CD40L mAb alone could induce the patient’s PBMCs to secrete markedly IgG. Our data suggest that the detection of CD40L expression on activated T cells may be used to identify sporadic cases of HIM as HIGM1.

Key words hyper-IgM syndrome, CD40L, CD40 mAb, cytokines

1 Introduction

Hyper-IgM syndrome (HIM) is a rare immunodeficiency characterized by an increased susceptibility to recurrent infections and markedly decreased serum IgG, IgA, and IgE, but with normal or more often elevated level of IgM [1-2]. X-linked, autosomal recessive, autosomal dominant, and acquired forms of the disease have been reported [3-5], indicating clinical and genetic heterogeneity. The X-linked form of the disorder (HIGM1) has been shown recently to be a result of mutation in the CD40 ligand (CD40L) gene [6-8].

CD40L is expressed by activated T cells. It has significant homology with TNF [9]. CD40L gene has been mapped to Xq 26.3-27.1 [9]. CD40L cDNA has an open reading frame of 785 bp long that codes for a type 1 transmembrane protein of 261 amino acids consisting of a 22 amino acids cytoplasmic domain, a 24 amino acids transmembrane domain, and a 215 amino acids extracellular domain [5]. The interaction between CD40L and B cell antigen CD40 is critical to B cell activation and immunoglobulin class switching [10-12]. Anti-CD40 monoclonal antibody (mAb) in synergy with IL-4 induces B-cell proliferation and IgE secretion [14-15] and in synergy with IL-10 induces B cell to produce IgG, IgA, and IgM [16-17]. To date, more than forty CD40L mutations have been identified [18]. These observed mutations including point mutations and deletions lead to premature stop codons and frame shifts in some cases. Most of the mutations are point mutations in the extracellular domain of CD40L which prevent it from binding to CD40.

We herein report a novel mutation in CD40L gene in a sporadic male case of HIM with no family history and the B cell function in response to anti-CD40 mAb and cytokines.

2 Materials and Methods

2.1 Patient
A 10-year-old Chinese boy (WMJ), referred to
Xin Hua Hospital in Shanghai because of recurrent infections occurring from the first year of life, with persistent neutropenia. The patient had severely reduced levels of IgG, IgA, and markedly elevated IgM (9.2 g/L). The percentage of CD3, CD4, CD8, CD19 positive cells in peripheral blood of the patient is 81.9%, 40.5%, 43.8%, 14.6% respectively. The patient received regular infusions of intravenous immunoglobulin. No disease-related information was obtained from family studies.

2.2 T cell activation and CD40L detection

PBMCs were prepared by centrifugation of heparinized venous blood over lymphocyte separation medium (Shanghai Second Reagents Factory). Mononuclear cells harvested from the interface were washed twice in phosphate buffered saline (PBS) and resuspended in RPMI 1640 (GIBCO, BRL) containing 10% FCS (GIBCO, BRL), and 100U/ml of penicillin/streptomycin at a concentration of 10^7/ml. T cells in PBMCs were stimulated with PMA (10 ng/ml) plus ionomycin (1 μg/ml) (Sigma) and incubated at 37°C for 6h in a 5% CO2 humidified incubator. Cells were subsequently washed, resuspended in PBS/2% FCS/0.1% NaN₃ (staining medium) at 10^7/ml. The cells (10^5) were stained for 1h at 4°C with anti-human CD40L mAb M90 (IgG1, 10 μg/ml), or M92 (IgG2a, 10 μg/ml) (a gift of Dr. Amitage, Immunex Corporation, Seattle). The cells were washed once with staining medium and resuspended in staining medium containing FITC-labelled goat antimouse IgG (final concentration of 1:20, Wuhan Institute of Biological Products) and incubated at 4°C for 30 min. The cells were subsequently washed once with staining medium and analyzed on a FACS can (Becton Dickinson, CA). Irrelevant mouse IgG1 or IgG2a antibody was used as isotype control.

2.3 Nested PCR and DNA sequencing

5 x 10^5/ml PBMCs from the patient and control were activated with PMA (10 ng/ml) and ionomycin (1 μg/ml) for 6h and total RNA was extracted. Reverse transcription of CD40L mRNA to cDNA was done according to standard method using Reverse Transcription System (Promega). 10% of the first strand of CD40L cDNA is used for the first PCR. The primer used was 5'-CTGCCAGAAGAT-ACCATT-3' and 5'-GGGGTTGCTGCTTCCAGAT-3' (nested 1: 94°C, 1', 52°C, 1.5', 73°C, 2', 27 cycles). Then, 1/50 of the first PCR product was used for the second PCR. The internal primer was 5'-CCATTTCAACTTTAACAC-3' and 5'-ACCGCTGTGCTATATT-3' (nested 2: 94°C, 1', 50°C, 1', 73°C, 1.5', 27 cycles). The second PCR product was cleaved by Hind III and run on a gel. After purification of PCR product by Wizard™ PCR Preps DNA Kit (Promega), sequencing was performed with Sequenase Kit (Gibco BRL).

2.4 Immunoglobulin secretion

PBMCs at a concentration of 10^5/ml in RPMI 1640 plus 10% FCS were stimulated with anti-CD40 mAb G28-5 (1 μg/ml) (kindly provided by Dr. E. A. Clark, School of Medicine, Univ. of Washington, Seattle), IL-2 (100 U/ml) (Sino-American Biological