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Research paper

Detection of one V_H antibody sequence in both healthy donors and urticaria patients

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Abstract

We have previously isolated anti-FcεRIα autoantibodies from phage libraries of healthy donors and urticaria patients. Strikingly, the same antibody, LTMα15, was isolated from both libraries. Sequence analysis revealed a germline configuration of the LTMα15 variable heavy (V_H) chain with a slightly mutated variable light (V_L) chain supporting its classification as a natural autoantibody. Distribution analysis of anti-FcεRIα autoantibodies by functional or serological tests delivered conflicting data. For this reason we have developed a new real-time PCR to analyse the distribution of LTMα15V_H in healthy donors and urticaria patients. Our new bioinformatic program permitted the design of a minor groove binder (MGB) *Taq*Man probe that specifically detected the LTMα15V_H. We were able to demonstrate a broad range of rearranged V_H gene copy number without any correlation to the state of health. Monitoring LTMα15V_H gene copy number in a single donor over a period of 70 days revealed a time-related fluctuation of circulating B cells carrying LTMα15V_H. We propose that our real-time PCR may serve as a model for the quantification of natural antibody sequences at a monoclonal level.

Keywords: Natural autoantibody; Germline configuration; Bioinformatics; Real-time PCR; Minor groove binder *Taq*Man probe

1. Introduction

Chronic idiopathic urticaria (CIU) is defined by the occurrence of daily wheals and itching for at least 6 weeks. The wheals, redness and itching of CIU are due to the release of histamine and other mediators from dermal mast cells. Evidence of the involvement of functional autoantibodies against the α-chain of the high-affinity IgE receptor (FcεRIα) of dermal mast cells and basophils in CIU was first reported by Hide et al. (1993, 1994). Such anti-FcεRIα autoantibodies were suggested to be a selective marker for a subset of urticaria patients (Fiebiger et al., 1995). However, we have previously reported the presence of anti-FcεRIα autoantibodies in the serum of healthy donors (Pachlopnik et al., 2004) as well as in multidonor intravenous IgG (IVIg) preparations (Horn et al., 1999). Furthermore we have isolated anti-FcεRIα autoantibodies (LTMα15, UMα16, UGα8) using phage display tech-

Abbreviations: V_H, variable heavy chain; V_L, variable light chain; CDR, complementary determining region; CIU, chronic idiopathic urticaria; FcεRIα, α-chain of the high-affinity IgE receptor; C_T, threshold cycle number; MGB, Minor groove binder; PBGD, porphobilinogen deaminase.

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nology (Horn et al., 2001; Miescher et al., 2001). LTMα15 was isolated from an IgM library constructed from children's tonsillar B cells. UMα16 and UGα8 originated from an IgM and IgG library, respectively, that were constructed from the B cells of urticaria patients. Comparison with the known germline sequence of human variable heavy (V_H) and variable light (V_L) chain segments (Tomlinson et al., 1992, 1995; Williams et al., 1996) indicated that LTMα15, UMα16 and UGα8 V_H chains showed 100% homology to the germline V_H3–23 sequence, whereas the λ light chains were slightly mutated and most similar to the V_λ germline λ6a. Furthermore, sequence analysis showed that LTMα15 and UMα16 are 100% identical. Due to the germline configuration of the heavy chains and the fact that the light chains are only slightly mutated, we suggest that LTMα15, UMα16 and UGα8 represent natural autoantibodies, which may be present in all humans. This evidence points to the ubiquitous presence of natural anti-FcεRIα autoantibodies but their role as a selective marker for urticaria patients remains unclear. Therefore, our goal was to investigate further the distribution of anti-FcεRIα autoantibodies in healthy donors and urticaria patients.

Up to now the distribution of anti-FcεRIα autoantibodies was analysed by functional and serological tests. However, no significant relation was found between autologous serum skin test positivity and serological detection of anti-FcεRIα autoantibodies by enzyme immunoassays (Sabroe et al., 2002; Pachlopnik et al., 2004). This discrepancy may occur due to the sensitivity of the performed tests. Here we describe the analysis of the distribution of the LTMα15V_H sequence by semi-nested RT–PCR which was shown to be two times more sensitive than a serological test to detect infectious agents (Pacheco et al., 2001). Additionally we have performed a real-time PCR. Quantitative analysis of the real-time PCR results enabled us to compare the relative gene copy number of LTMα15V_H sequence in healthy donors and urticaria patients. Because of the presence of V sequences highly similar to the LTMα15 V_H and V_L sequence in the V repertoire we have written a new bioinformatic program, which predicts potential primer annealing sites in any sequence. Using this program, we were able to determine a potential primer annealing site, which is present exclusively in the LTMα15V_H sequence. To this annealing site we have designed a 3′ end primer for the RT–PCR or a minor groove binder (MGB) probe to perform the real-time PCR. To the best of our knowledge, this is the first report describing the detection of a single natural immunoglobulin sequence in different donors. Our approach may therefore serve as a model for the detection and quantification of antibody sequences coding for natural antibodies.

2. Material and methods

2.1. DNA, RNA extraction, cDNA synthesis and plasmid DNA

Mononuclear cells of peripheral blood from 26 unselected healthy donors, 11 urticaria patients and of 25 cord blood samples were purified by Ficoll density gradient centrifugation (Lymphoprep, Pharmacia, Dübendorf, Switzerland). Total RNA was extracted using RNeasy Mini Kit (Qiagen AG, Basel, Switzerland). RNA was transcribed into cDNA using a primer specific for the first constant domain of the IgM gene (5′GCTCACACTAGTCTAGGCAATCACTGGAAGAGG 3′) and the Superscript TM II reverse transcriptase system (Invitrogen, Life technology, Basel, Switzerland). Genomic DNA was isolated using QIAamp DNA blood Maxi kit (Qiagen AG, Basel, Switzerland). Plasmid DNA containing the anti-FcεRIα antibody LTMα15 heavy chain sequence was produced in our laboratory as described (Miescher et al., 2001).

2.2. Identification of specific primer annealing sites for semi-nested RT–PCR

Specific primers were designed in order to detect the LTMα15 sequence in healthy donors and urticaria patients using PCR. The sequences of LTMα15 V_H and V_L are available at EMBL GenBank under the numbers AJ276097 and AJ276098. We intended to design a forward primer annealing either in the complementary determining regions (CDR) 1 or CDR2 and a reverse primer hybridising to the CDR3 of LTMα15 variable light (V_L) and variable heavy (V_H) chains. To identify specific primers, the CDR sequences of V_H and V_L of LTMα15 were independently compared to 82,566 different immunoglobulin sequences (ImMunoGeneTics database, University of Montpellier, France and French National Centre for Scientific Research: CNRS, France (Ruiz et al., 2000)) applying a BLAST search (Basic Local Alignment Search Tool, version 2.2.8). BLAST was used with gap open and extension penalties of 5 and 2, respectively. The *E*-value threshold was set to 1000. For comparison, sequences of CDRs of LTMα15V_H and V_L, which were shorter than the desired primer length of 23 nucleotides, were extended to 23 nucleo-