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SHORT COMMUNICATION

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Genetic polymorphism of the H1 subunit of inter-alpha-trypsin inhibitor

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Abstract Inter-alpha-trypsin inhibitor (ITI) consists of 3 subunits (H1, H2 and L) which are encoded by 3 distinct genes. We attempted to prepare the subunit-specific monoclonal antibodies to identify the subunit carrying ITI polymorphism. Three monoclonal antibodies which were specific against each of these 3 subunits (ITI H1, ITI H2 and ITI L) were selected. ITI types could be detected by anti ITI H1 antibody, but not by anti ITI H2 or anti ITI L antibodies. From the result, it was proved that ITI polymorphism originates from the ITIH1 gene products, therefore we propose to call this system ITI H1 polymorphism.

Key words Inter-alpha-trypsin inhibitor · ITIH1 · Polymorphism · Monoclonal antibodies

Introduction

ITI is composed of 3 subunits (H1, H2 and L) connected by a glycosaminoglycan (GAG) chain which can be digested by chondroitinase ABC [1]. ITI H1, H2 and L subunits are encoded by chromosome 3, 10 and 9, respectively [2]. Restraction fragment length polymorphisms (RFLPs) of ITI genes were reported [3–7], and the ITI polymorphism of serum protein was first described by Vogt and Cleve [8]. The relationship between the RFLPs of ITI genes and ITI polymorphism of serum protein are not clear. ITI phenotypes have been detected by immunostaining using polyclonal anti ITI antibody. Until now the alleles *ITI*1*, *ITI*2*, *ITI*3*, *ITI*4*, *ITI*5*, *ITI*6*, *ITI*T* and *ITI*Y* have been reported [8–12] and recently, Vogt et al. [13] reported that ITI protein polymorphism may be controlled by the gene coding for the ITI heavy chain H1.

In the present work, we tried to determine the ITI subunit carrying the ITI polymorphism using subunit-specific monoclonal antibodies.

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Materials and methods

Preparation of monoclonal antibodies

BALB/c mice 1.5 months old were immunized by an intraperitoneal injection with 50 μg of the purified ITI [14] emulsified in Hunter's TiterMax adjuvant (CytRx, USA). The mice were given another injection 2 weeks later. A booster injection with 50 μg of the purified ITI was given 2 weeks after the second injection. Three days after the booster injection, cell fusion was made according to Gefter et al. [15] and the hybridomas producing antibodies were cloned twice by limiting dilution. The specificity of monoclonal antibodies against each ITI subunit was determined by Western immunoblotting following SDS-PAGE in the presence and absence of 2-mercaptoethanol. SDS-PAGE was performed by the method of Laemmli [16]. The cell-free culture supernatant from 3 selected hybridomas was collected and used without further purification. The isotype of monoclonal antibodies was determined by ISOTYPE Ab-STAT (Sang Stat Medical, USA).

ITI phenotyping was performed using polyacrylamide gel isoelectric focusing (IEF) and immunoblotting procedures [12].

Results and discussion

A genetic polymorphism of ITI was analysed at the protein level. To determine the subunit carrying the ITI polymorphism, we made 3 monoclonal antibodies against each of the 3 ITI subunits, ITI H1, ITI H2 and ITI L. The specificity of each of them was determined by SDS-PAGE followed by Western immunoblotting. In the presence of 2-mercaptoethanol, these 3 monoclonal antibodies reacted to the main ITI band (230 kDa) in the same manner as the polyclonal anti-ITI antiserum (Dako, Denmark). After chondroitinase ABC digestion of ITI, the bands of 170, 86, 79 and 23 kDa interacted with polyclonal anti-ITI antiserum (Fig. 1A). These 4 bands were identified as ITI H1+H2, ITI H2, ITI H1 and ITI L, respectively [14, 17]. One of the monoclonal antibodies interacted with 2 bands which corresponded to ITI H1+H2 and ITI H1 (Fig. 1B). Another monoclonal antibody interacted with 2 bands which corresponded to ITI H1+H2 and ITI H2 (Fig. 1C). The other monoclonal antibody detected one band corresponding to ITI L (Fig. 1D). Therefore each of these 3

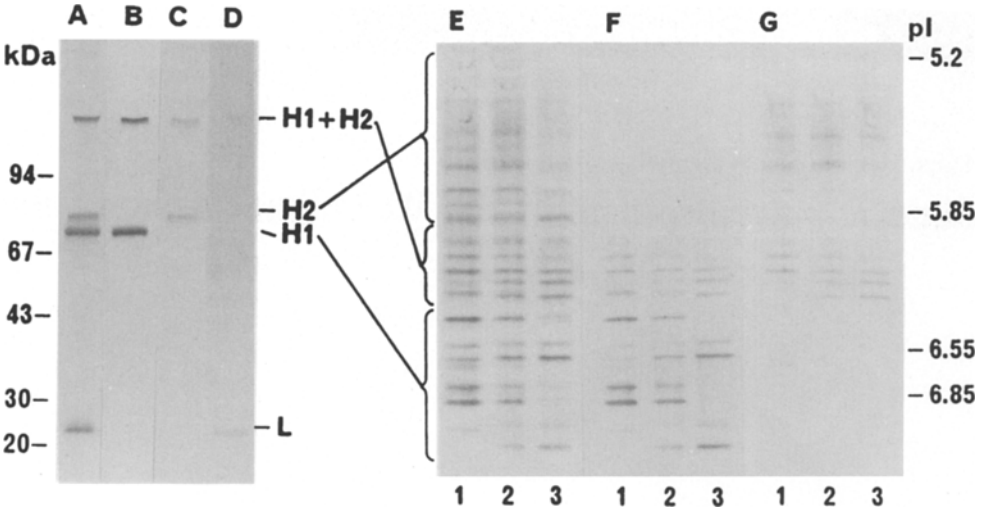

Fig. 1 Immunoblotting band patterns of ITI after SDS-PAGE (A–D) and IEF (E–G). Chondroitinase ABC treated samples were analysed by SDS-PAGE (4–20% gradient gel) in the presence of 2-mercaptoethanol. Sialidase and Chondroitinase ABC treated samples were analysed by IEF in the pH range 4.5–8. ITI bands were detected with polyclonal anti-ITI (A, E), monoclonal anti-ITI H1 (B, F), monoclonal anti-ITI H2 (C, G) and monoclonal anti-ITI L (ED) antibodies. The isoelectric point of ITI L was approximately 5.0. The phenotypes are: lane (1) ITIH1 1; (2) ITIH1 2–1; (3) ITIH1 2

monoclonal antibodies was found to be specific against ITI H1, ITI H2 and ITI L, respectively. The specificities of these monoclonal antibodies were also confirmed by the band mobilities of ITI H1 and ITI H2 which were reversed in the absence of 2-mercaptoethanol [18].

The isotypes of monoclonal anti-ITI H1, anti-ITI H2 and anti-ITI L antibodies were IgG1, IgG1 and IgG2a, respectively.

In Fig. 1, E–G show immunoblotted band patterns after IEF of sialidase and chondroitinase ABC-treated samples using polyclonal anti-ITI antiserum and monoclonal anti-ITI H1 and anti-ITI H2 antibodies, respectively. ITI phenotyping can be done both by the monoclonal anti-ITI H1 antibody (Fig. 1F) and by the polyclonal anti-ITI antiserum (Fig. 1E). On the other hand, ITI phenotyping cannot be defined by the monoclonal anti-ITI H2 antibody or by the monoclonal anti-ITI L antibody. The monoclonal anti-ITI L antibody recognized a single band near the sample application area which had a pI value less than 5.2. The band locations of ITI H1, ITI H1+H2, ITI H2 and ITI L in IEF patterns were demonstrated by immunoblotting patterns using each of these 3 monoclonal antibodies. This was also confirmed by the analysis of 2 dimensional electrophoresis (data not shown).

Accordingly, the present investigation revealed that ITI phenotypes can be recognized as a polymorphism of ITI H1 subunit. This result corresponded well with the result obtained at the DNA level by Vogt et al. [13]. Therefore we propose to call this system ITIH1 polymorphism.

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