PRESENCE OF ANTI-DESMOGLEIN 3 AUTO-ANTIBODIES IN HEALTHY RELATIVES OF IRANIAN PATIENTS WITH PEMPHIGUS VULGARIS

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ABSTRACT

Pemphigus vulgaris (PV) is an autoimmune blistering disease of the stratified squamous epithelia. It is caused by an autoantibody against Desmoglein 3 (Dsg 3), a normal component of desmosomes which has a critical role in the attachment of keratinocytes. Several studies have demonstrated that healthy relatives of patients with autoimmune diseases including PV are more susceptible to produce autoantibodies or develop autoimmune diseases. This susceptibility to autoimmunity or autoimmune diathesis has been shown to be an autosomal dominant trait. In this study the anti-Dsg 3 autoantibody was found in the sera of 35% of 46 healthy relatives of Iranian patients with PV with immunoblot assay. This shows that the genetic susceptibility to autoimmunity is present in the family members of patients with PV and it is mostly transmitted as an autosomal dominant trait.

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INTRODUCTION

Pemphigus vulgaris (PV) is a rare blistering disease of the skin and mucous membranes. PV is considered an organ specific autoimmune disease[1]. It is caused by an IgG autoantibody against Desmoglein 3 (Dsg 3), a normal component of the desmosomes. This 130 kD molecule belongs to the cadherin superfamily of adhesion molecules and has an important role in the attachement of keratinocytes[2]. The autoantibody binds to this molecule which results in loss of its function, separation of the keratinocytes and development of the blisters.

The anti-Dsg3 autoantibody has also been found in the serum of healthy relatives of the patients in low titers[3,4]. The presence of this antibody was strongly associated with certain major histocompatibility complex (MHC) haplotypes and appeared to be inherited as an autosomal dominant trait[3,4]. In this study the healthy relatives of Iranian patients with PV were screened for the presence of autoantibodies against human epidermis.

MATERIALS AND METHODS

A total of 46 healthy first degree relatives (parents, siblings or children) of 21 Iranian patients with PV were studied for the presence of autoantibodies against human epidermis. The diagnosis of PV was confirmed by routine histologic examination and immunoblotting in all patients.

Blood samples were obtained from the relatives and after centrifugation the serum was collected and stored at -20C until being used. The antigenic specificities of the sera was studied by an immunoblot assay according to the previously described method[3]. Briefly an epidermal extract was prepared from normal human skin. This extract was passed successively through Sepharose 4B columns coupled with normal human serum and human IgG in order to block nonspecific binding sites. SDS polyacrylamide gel electrophoresis was carried out on the extract along with standard molecular weight

markers in 8% running gel with 3% stacking gel. Then the gel was immersed in blotting buffer for 30 minutes and electrophoretically transferred to a nitrocellulose membrane (Millipore, Bedford, Mass). The membrane was washed with phosphate buffered saline (PBS) for 10 minutes and incubated with 3% bovine serum albumin (BSA) for 2 hours at room temperature. Then the membrane was washed 3 times with PBS, 10 minutes each, and cut into 2.5 millimeter wide strips.

Sera of the study subjects were diluted to 1/20 with 3% BSA in PBS. This dilution was chosen based on the results of previous studies[3]. The strips were incubated face up with 5 milliliters of diluted sera and positive and negative controls in the slots of a slotted incubation tray for 2 hours at room temperature and the washed three times with PBS, 5 minutes each. The strips were incubated with peroxidase conjugated goat anti-human IgG diluted 1/3000 with 3% BSA in PBS for 2 hours at room temperature. Finally the strips were washed several times with PBS and attached to IBI enzygraphic web (Eastman Kodak, New Haven, CT). As the color developed by the web is unstable, photocopies were taken from the strips immediately.

RESULTS

Sera of 16 (35%) of 46 relativeds of patients with PV contained autoantibodies against a 130 kD molecule i.e. Dsg 3. No autoantibodies could be detected in the sera of 30 of the relatives. This is compatible with an autosomal dominant transmission of the genetic susceptibility to produce autoantibodies against Dsg 3.

DISCUSSION

It has been demonstrated that relatives of patients with autoimmune diseases are more susceptible to the development of the same or more frequently other autoimmune diseases. Also they may produce various autoantibodies which are not pathogenic. This susceptibility to autoimmunity have been found in the relatives of patients with several autoimmune diseases such as SLE(5), rheumatoid arthritis[6], autoimmune thyroid diseases[6,7,8], IDDM[8,9], Sjogren's syndrome[10], dermatitis herpetiformis [11], autoimmune hemolytic anemia and idiopathic thrombocytopenic purpura[12]. It seems that certain families are more prone to autoimmunity. So it has been suggested that an "autoimmune diathesis" may be present in these families[13]. This concept is similar to the atopic diathesis in which allergies are more frequent in certain families, but the specific clinical expression or phenotype of this susceptibility varies from one person to another in the same family.

At least two phenomena may be involved in the development of autoimmune disease[13]. The first is an inherent nonspecific predisposition to autoimmunity i.e., the autoimmune diathesis. In many studies the inheritance of this susceptibility has been compatible with an autosomal dominant trait without any association with MHC alleles[10,13]. The second phenomenon is the development of an organ specific autoimmunity as an autoantibody (in the absence of clinical disease) or a clinical autoimmune disease[13]. The differences between nonpathogenic and pathogenic autoantibodies have been studied but are not completely determined.

It seems that both phenomena exist in PV. The family members of patients with PV were found to be more susceptible to the development of other autoimmune diseases[14]. It shows that the generalized susceptibility to autoantimmunity or autoimmune diathesis is present in these families.

The second phenomenon is also present in families of patients with PV. The anti-Dsg 3 autoantibody is present in the sera of some of the healthy relatives of the patients. The titer of this autoantibody was much lower in the relatives compared to the patients[3,4]. The presence of the autoantibody appeared to be inherited as an autosomal dominant trait. The significance of these nonpathogenic antibodies is not clear. It has been suggested that these autoantibodies are components of a natural autoantibody system and control the immune responses via the idiotype-anti-idiotype network[15].

The present study confirms that the healthy family members of patients with PV can produce low titers of anti-Dsg 3 autoantibody. The production of this antibody is mostly compatible with autosomal dominant mode of transmission. At a later stage

unknown genetic or environmental factors may trig ger the production of high titers of the autoantibody and some changes in its characteristics which con- verts it into a pathogenic antibody. This will result in the development of the clinical disease in these genetically predisposed individuals.

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