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Viewpoint

Is Grover's disease an autoimmune dermatosis?

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Abstract: Grover's disease (GD) is a transient or persistent, monomorphous, papulovesicular, asymptomatic or pruritic eruption classified as non-familial acantholytic disorder. Contribution of autoimmune mechanisms to GD pathogenesis remains controversial. The purpose of this study was to investigate antibody-mediated autoimmunity in 11 patients with GD, 4 of which were positive for IgA and/or IgG antikeratinocyte antibodies by indirect immunofluorescence. We used the most sensitive proteomic technique for an unbiased analysis of IgA- and IgG-autoantibody reactivities. Multiplex analysis of autoantibody responses revealed autoreactivity of all 11 GD patients with cellular proteins involved in the signal transduction events regulating cell development, activation, growth, death, adhesion and motility. Semiquantitative fluorescence analysis of cultured keratinocytes pretreated with sera from each patient demonstrated decreased intensity of staining for desmoglein 1 and/or 3 and PCNA, whereas 4 of 10 GD sera induced BAD expression, indicating that binding of autoantibodies to keratinocytes alters expression/function of their adhesion molecules and activates apoptosis. We also tested the ability of GD sera to induce visible alterations of keratinocyte shape and motility *in vitro* but found no specific changes. Thus, our results demonstrated that humoral autoimmunity in GD can be mediated by both IgA and IgG autoantibodies. At this point, however, it is impossible to conclude whether these autoantibodies cause or are caused by the disease. Antidesmoglein antibodies may be triggered by exposure to immune system of sequestered antigens due to disintegration of desmosomes during primary acantholysis. Clarifying aetiology of GD will help improve treatment, which currently is symptomatic and of marginal effectiveness.

Key words: autoantibody – Grover's disease – keratinocytes – proteomics

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Introduction

Grover's disease (GD) is a monomorphous, papulovesicular, asymptomatic or pruritic eruption described by Ralph W. Grover in 1970 (1). GD is currently classified as a non-familial, non-immune-mediated, acantholytic disorder (2). It has been proposed that GD is a syndrome caused by various aetiologies resulting in the same clinical manifestations (3). GD may have transient eruptive, persistent pruritic and chronic asymptomatic courses (4). Distinct patterns of acantholysis and dyskeratosis in GD encompass the histopathological features of Hailey–Hailey disease, pemphigus vulgaris and foliaceus, and Darier's disease (2).

The issue of contribution of the (auto)immune mechanisms to GD pathogenesis remains controversial. While the results of both direct and indirect immunofluorescence (IIF) are usually negative or inconsistent, it has been hypothesized that GD represents a host inflammatory response to altered self-antigens recognized by the immune system as foreign (3) and that there may be a subgroup of GD in which immune factors are involved through a variety of different mechanisms (5–7). Several classic immunological studies of skin biopsies from patients with GD demonstrated deposition of IgA, IgG, IgM and C3. Both IgG antibodies and C3 were observed both at the dermal–epidermal junction, like in pemphigoid (6–10), and within the epidermis, producing a pemphigus-staining pattern (7,11–13). Biopsy specimens from involved skin also demonstrated IgM at the basement membrane zone and in the walls of superficial dermal vessels, and IgA, C3 and fibrin in the walls of papillary blood vessels (6). IgA and IgM were also found in cytoid bodies (6). Correspondingly, IIF studies revealed circulating antibodies to the suprabasal keratinocyte, basal cell and/or basement membrane zone antigens (7). It was opined, however, that positive immunofluorescence might represent minimal variants of pemphigus or yet unclassified condition (14), or an epiphenomenon (15,16). An indirect support of the hypothesis that GD is an autoimmune disorder is provided by the association of GD with the autoimmune diseases pemphigus and pemphigoid (17–19), as well as by the therapeutic effect of rituximab, which inhibits antibody production (20).

The purpose of this study was to further characterize antibody-mediated autoimmunity in patients with GD.

Results and discussion

The study included 11 patients diagnosed with GD based on the results of comprehensive clinical and histopathological examinations (Table 1). To determine whether patients' sera contain autoantibodies against cutaneous self-antigens, we performed IIF experiments. The specimens of commercially obtained normal human skin were preincubated for 24 h with serum from patients with GD versus normal human serum, and stained with FITC-labelled anti-human IgA, IgE, IgG or IgM antibodies. Four of eleven serum samples (33.4%) were positive for antikeratinocyte antibodies, one of which contained IgA autoantibodies, two IgG autoantibodies and one sample contained both IgA and IgG antibodies (Table 1). The fraction of seropositive sera among our patients with GD was very similar to that reported by Dr. Bystryn (7). The seropositivity of our patients with GD did not correlate

Table 1. Characteristics of patients with Grover's disease (GD) involved in this study

Patient #	Age at diagnosis (year old)	Approximate duration of disease	Itch	Treatment prior to blood draw	Histopathological pattern	IIF results
1	88	1 year	Present	None	PV-like	Negative
2	54	1.5 years	Present	0.25% desoximetasone cream, 4% pilocarpine gel	HH-like	Negative
3	55	4 months	Present	None	D-like	Negative
4	84	16 years	Present	0.05% fluocinonide cream, niacinamide 500–1000 mg/day	HH-like	IgA (1/1280), IgG (1/5120)
5	51	4 months	Present	None	PV-like	IgG (1/1280)
6	63	1 year	Present	0.05% clobetasol cream, 0.25% desoximetasone cream, 4% pilocarpine gelfluconazole 100 mg/day × 14 days	D-like	IgG (1/2560)
7	86	2.5 months	Present	Cephalexin 250 mg/day × 10 days	D-like	IgA (1/40)
8	69	2 years	Present	None	PF-like	Negative
9	52	4.5 months	Absent	None	D-like	Negative
10	70	14 years	Present	0.025% triamcinolone cream, niacin 400 mg/day	HH-like	ND
11	53	19 years	Present	0.05% fluocinonide cream, 4% pilocarpine gel, 1% pimecrolimus cream, 0.03% tacrolimus ointment, bethanechol 30 mg/ dayniacinamide 1500 mg/day	D-like	ND

D, Darier's disease; HH, Hailey–Hailey disease; IIF, indirect immunofluorescence on human skin; ND, not done; PF, pemphigus foliaceus; PV, pemphigus vulgaris.

with either duration of their disease or the histopathological pattern.

It is well recognized that multiplex analysis of autoantibody responses against a spectrum of candidate antigens represents a powerful screening tool allowing elucidation of the overall autoimmune process rather than individual components (21). Our recent studies of patients with pemphigus vulgaris have vividly demonstrated that proteomic technologies enable a large-scale characterization of immune responses against the autoantigens that may be involved in the development and progression of cutaneous autoimmune disease (22,23). Therefore, we used the proteomic microarray for an unbiased analysis of IgA and IgG autoantibody profiles of 11 patients with GD. The microarrays identified a unique collection of antigens differentially reacting with GD sera (Fig. 1). This outcome did not contradict results of our IIF studies, because antigen arrays proved to be four- to eightfold more sensitive than conventional detection of autoantibodies (24).

The top 15 differentially reactive antigens included proteins that mediate signal transduction events and play a role in the regulation of cell development, activation, growth, adhesion and motility (Fig. 1) Notably, both IgA and IgG antibodies recognized peripheral myelin protein 22 (PMP-22) with high specificity. The IgA antibodies also targeted a member of PMP-22/gas3 family termed PERP (p53 apoptosis effector related to PMP-22). These proteins are also targeted in autoimmune pemphigus (22,23). The pathophysiological significance of these autoantibodies is suggested by the fact that PERP knockout mice display pemphigus-like acantholysis caused by dissolution of desmosomes (25). In concurrence, the ultrastructural analysis of GD skin reveals rarefaction of desmosomes and widening of intercellular spaces (26). Furthermore, PERP is involved in the extrinsic apoptotic pathway, playing a 'death receptor' role (27).

The proteomic analysis of sera from our patients with GD also revealed the significantly different reactivities with members of the cell adhesion molecule, the nicotinic acetylcholine receptor, the growth factor receptor and the major histocompatibility complex protein families as well as several mitochondria-associated proteins and the Fc fragment of IgG (Fig. 1). These protein families are

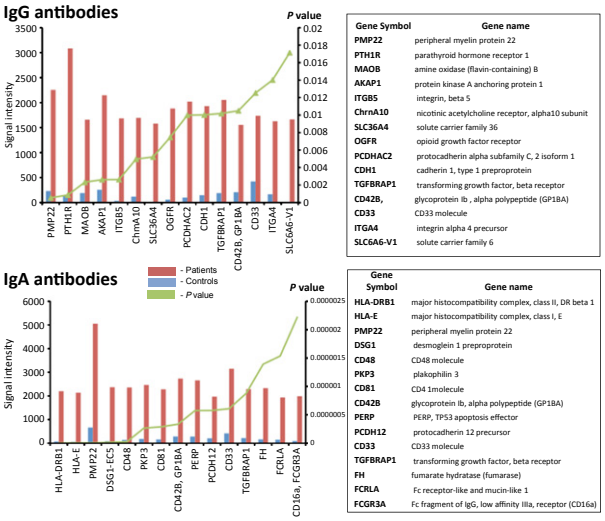

Figure 1. The signal intensities of differentially reactive proteins recognized by IgA and IgG antibodies from patients with Grover's disease (GD). The sera from 11 patients with GD and 43 healthy donors (controls) were probed in the multiplexed protein array platform containing 701 human genes encompassing the keratinocyte proteins printed on the chip manufactured by us in a previous study (22). The genes were amplified and cloned using high-throughput PCR and recombination method, employing the gene-specific primers containing 20-bp nucleotide extension complementary to ends of linear pXT7 vector. The recombinant plasmids were isolated, labelled with haemagglutinin at 3′ end and polyhistidine at 5′ end and printed onto nitrocellulose-coated glass slides. Duplicates of test sera preabsorbed with *Escherichia coli* lysate were applied to arrays overnight at 4°C. Differentially reactive proteins between groups were determined using a Bayes regularized t-test adapted from Cyber-T for protein arrays, as detailed by us elsewhere (23). The data were analysed using Student's *t*-test, and the Benjamini and Hochberg (BP) method to control for the false discovery. The graphs represent 15 top antigens recognized by IgA and IgG autoantibodies sorted by the ascending *P* values.

also targeted in autoimmune pemphigus [reviewed in (28)]. Some other muscarinic and nicotinic receptor proteins as well as adhesion molecules were also recognized by GD sera, but signal intensity did not reach statistical significance (not shown). One of us, DP, has recently reported correlation of GD clinical activity with an intake of varenicline, a drug that alters signalling from nicotinic acetylcholine receptors (29). Targeting of the same signalling pathways by autoantibodies may, therefore, account for similarities of the acantholytic patterns between GD and pemphigus.

Proteomic results suggested that GD autoantibodies contributed to a loss of keratinocyte adhesion, abnormal growth and keratinization manifested by acantholysis and dyskeratosis. These findings are in keeping with the recent results showing that adhesion molecules, such as desmoglein (Dsg) 1 targeted by GD sera, are functionally associated with regulation of keratinocyte proliferation and differentiation (30). Indeed, it is well known that abnormal keratinization is one of the phenotypic manifestations of mutations of the DSG1 gene [reviewed in (31)]. Furthermore, anti-Dsg 1 autoimmunity mediated by IgA and IgG antibodies is associated with appearance of dyskeratotic cells in the epidermis (32). In Darier's disease, acantholysis does not directly initiate dyskeratosis (33), and appearance of dyskeratosis can be attributed to precocious keratinization (34). The process of dyskeratosis in the Darier's disease-like form of GD may therefore be more complex and involve genetically determined and/or autoantibody-mediated dysregulation of keratinization as well as activation of the apoptotic pathways, as seen in skin lesions of patients with GD (35).

To test the hypothesis that in addition to cell–cell adhesion, keratinocyte proliferation and programme cell death are also affected by GD autoantibodies, we analysed by semiquantitative immunofluorescence the expression of well-established markers of keratinocyte cell-to-cell adhesion (Dsg 1 and 3), proliferation (PCNA) and apoptosis (BAD) in monolayers of normal human epidermal keratinocytes preincubated with GD sera. We found a significantly ($P < 0.05$) decreased intensity of staining for one or both Dsg molecules, compared with the staining of keratinocytes exposed to normal control serum (Fig. 2). These findings are in keeping with results of classic immunohistochemical studies that showed the loss of adhesion molecules from the desmosomes of acantholytic cells in GD lesions (36–38). Exposure to 9 of 10 tested GD sera also decreased PCNA expression, whereas 4 sera elevated BAD, suggesting that autoantibody binding activates keratinocyte apoptosis, perhaps, *via* the PERP-mediated pathway.

It is broadly recognized that acantholysis and apoptosis of keratinocytes in autoimmune pemphigus develop in parallel, *via*

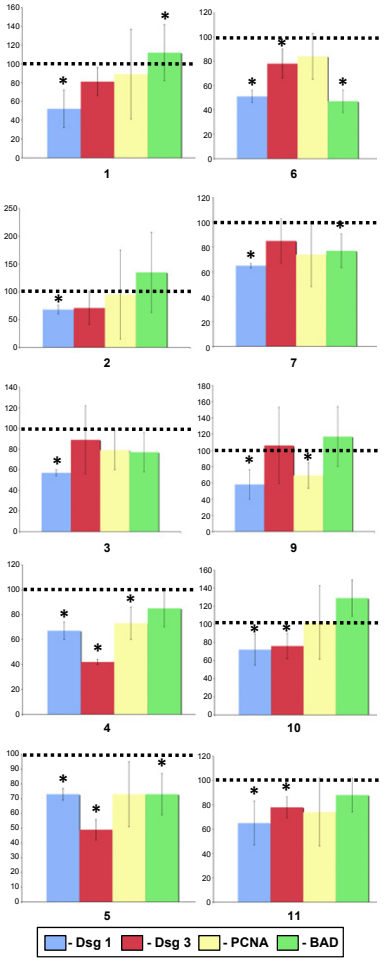

Figure 2. Effects of sera from patients with Grover's disease (GD) on the expression of the keratinocyte cell-to-cell adhesion, proliferation and apoptosis markers. Normal human neonatal keratinocytes were grown on cover slips in serum-free medium to confluence, preincubated for 24 h with 20% of heat-inactivated sera from 10 patients with GD (not done for patient #8) versus normal human serum purchased from Bioreclamation, Inc. (Westbury, NY) as control. The monolayers were washed, fixed with 4% paraformaldehyde and incubated with antibodies to Dsg 1 (1:200; R&D systems, Minneapolis, MN), Dsg 3 (1:200; R&D systems), PCNA (1:200; Abcam, Cambridge, MA) or BAD (1:500; Novus Biologics, Littleton, CO) followed by secondary, FITC-labelled antibodies. The pattern of staining was nuclear for PCNA, cytoplasmic for BAD and pemphigus-like for Dsg 1 and 3. The protein expression was analysed by semiquantitative fluorescence by visualizing three fields in each slide and then measuring the mean intensity of fluorescence at the magnification 20× (*Y*-axis). The fluorescence intensity in control samples was taken as 100% (dashed line). *$P < 0.05$ compared with control.

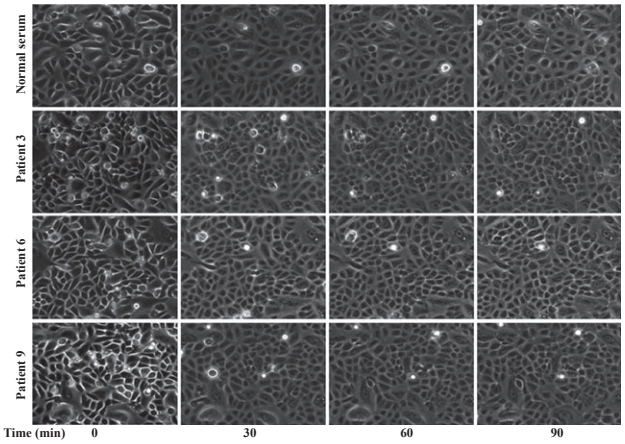

Figure 3. The sera from patients with Grover's disease (GD) do not induce acantholysis in keratinocyte monolayers. Confluent monolayers of human keratinocytes grown in 6-well plates were fed the growth medium containing 20% of heat-inactivated sera of nine patients with GD or normal individuals (controls) and subjected to time-lapse microscopy over the course of 3 h on an inverted phase-contrast computer-linked microscope in a temperature (37°C) controlled environment. The photographs from the video-recording demonstrate that cell morphology did not appear to be altered by the GD compared with the control sera. The images from the experiments with the three GD sera shown herein are representative of those obtained in experiments with the other tested GD sera.

the process termed apoptolysis (39) and that pemphigus autoantibodies cause keratinocyte shrinkage followed by disruption of desmosomal bridges. The later phenomenon provided the basis for the basal cell shrinkage hypothesis of pemphigus acantholysis (40). To test the hypothesis that GD autoantibodies cause acantholysis by altering cell shape, we performed a time-course study of live keratinocytes exposed to patients' sera. Although immediately following the addition of patients' and normal sera, keratinocytes flattened, over the course of 3-h video-recording, the cell–cell contacts remained intact, and the cytoplasm motility did not appear to be different between experimental and control keratinocytes (Fig. 3). Perhaps, cultured normal human keratinocytes did not exhibit any morphological abnormalities upon exposure to GD sera because they were not sensitive to GD antibodies.

The fact that GD sera did not alter keratinocyte morphology also suggests that the mechanism of acantholysis in GD differs from that in autoimmune pemphigus. The principal difference may result from activation, or inhibition, of distinct downstream signalling pathways by antikeratinocyte antibodies characteristic of GD versus those of pemphigus. In pemphigus vulgaris, for example, a constellation of pathogenic antibodies, in addition to Dsg 3 and acetylcholine receptors, includes antibodies to several other adhesion molecules, for example desmocollins, and the Ca^{2+}/Mn^{2+}-ATPase encoded by the ATP2C1 gene (22), which is known to be mutated in the acantholytic genodermatosis termed Hailey–Hailey disease or benign familial pemphigus (41). Alternatively, the primary defect may be not in cell adhesion, but in the process of keratinization manifested by the dyskeratosis in GD lesions.

Conclusions

Our results provide an insight into the mechanism of keratinocyte damage in patients with GD. While this study was focused on the autoimmune aspects of GD, the aetiology and pathogenesis of GD remain elusive. The common triggers of GD, such as heat, sweating, febrile illnesses, immunodeficiency, skin cancer and haematologic malignances as well as its coexistence with numerous other dermatoses [reviewed in (2–4)] indicate on the polypathogenic nature of this disease. Autoimmunity in GD can be mediated by both IgA and IgG autoantibodies. Admittedly, the results reported in this study are preliminary and additional studies should be performed to extrapolate our findings to GD pathophysiology. At this point, it is impossible to conclude whether these autoantibodies are the cause of or being caused by the disease. The latter scenario can be exemplified by the development of some anti-Dsg antibodies secondary to exposure of sequestered antigens to immune system during disintegration of desmosomes in the course of acantholysis (42). The small sample size precluded us from establishing the relationship of the autoantibody levels and antigen specificities with disease severity, natural course (transient versus persistent) and histopathological pattern of GD. These important issues may be addressed in future studies. Future studies may test a hypothesis that acantholysis in patients with GD results from both the presence of antikeratinocyte antibodies and increased sensitivity (abnormality) of epidermal keratinocytes in patients' skin. Clarifying aetiology of GD will help improve treatment, which currently is symptomatic and of marginal effectiveness. There may be a subset of patients with GD producing pathogenic antibodies who would benefit from an immunosuppressive therapy.

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Conflict of interests

The authors have declared no conflicting interests.

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