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Update on the cloning of monoclonal anti-desmoglein antibodies from human pemphigus patients: implications for targeted therapy

John R. Stanley, Ken Ishii, Don L. Siegel, and Aimee S. Payne

University of Pennsylvania School of Medicine, Philadelphia, PA, USA

Abstract

Autoantibodies in pemphigus foliaceus (PF) and vulgaris (PV) bind to desmoglein (Dsg) 1 and 3, respectively, and cause loss of keratinocyte adhesion. To characterize the pathogenicity and genetics of such antibodies we have used phage display to isolate monoclonal antibodies (mAbs) from patients. PCR is used to clone the heavy and light chain variable region of the peripheral B cells into a vector that creates a phage particle with the antibody expressed on its surface and the cDNA encoding that antibody inside. The library of phage produced from a PF or PV patient are then panned on a plate containing Dsg1 or Dsg3 to isolate clones. The cDNA of each clone is sequenced to characterize the genetics of the expressed mAb. The mAb from each unique clone is tested for pathogenicity either by injecting into normal human skin organ culture or into neonatal mice. Pathogenic antibodies cause typical pemphigus blisters. In both PV and PF patients the heavy chain (VH) genes used for Dsg-binding antibodies are severely restricted. PV and PF patients have both pathogenic and non-pathogenic mAbs. The immunochemical characteristics of the antibodies (including pathogenicity) sort with the VH, not the VL, gene. These monoclonal pathogenic antibodies can be used to screen peptide libraries to find short peptides that block antibody binding. In summary, the antibody response is restricted and, therefore, it may be feasible to target the specific pathogenic antibodies for therapy.

Introduction

Autoantibodies in pemphigus foliaceus (PF) and pemphigus vulgaris (PV) bind to desmoglein (Dsg) 1 and 3, respectively, and cause loss of keratinocyte adhesion with resultant blister formation.^{1–6} To characterize the pathogenicity and genetics of such antibodies we have used phage display to isolate monoclonal antibodies (mAbs) from patients.^{7–8} Phage display has been used to gain insight into other autoantibody-mediated diseases such as idiopathic thrombocytopenic purpura.⁹ By cloning such monoclonal antibodies from pemphigus patients it may be possible to address some of the following issues:

Do pemphigus patients have both pathologic and non-pathologic antibodies?

Before antibody cloning from pemphigus patients, it was known that IgG from patients' sera caused blisters.^{10–12} Because such IgG is a mixture of different antibodies against Dsg it was not clear whether individual antibodies alone are capable of causing disease; whether only a subset of antibodies is pathogenic; and whether a single clonal antibody can cause disease or if multiple antibodies binding different targets on Dsg are necessary.

Correspondence: J.R. Stanley, University of Pennsylvania School of Medicine, Philadelphia, PA, USA. jrstan@mail.med.upenn.edu.

Conflict of Interest

None declared.

Is the pathogenic antibody response genetically restricted?

For example, phage display has indicated that autoantibodies from idiopathic thrombocytopenic purpura patients almost all used only one variable heavy chain gene.⁹ If pathogenic antibodies from different pemphigus patients use one, or just a few, variable heavy chain (VH) genes then these specific chains could be potentially targeted for therapy. Antibodies from pemphigus patients must be cloned to determine their VH gene usage and its association with pathogenicity.

Cloning pemphigus antibodies will allow characterization of the pathologic idiotypes of the antibodies and the epitopes on Dsg to which they bind. In turn this allows determination if such epitopes and idiotypes are shared among different patients. In addition, cloning individual mAbs from patients permits one to determine if a single mAb can bind both Dsg3 and Dsg1 and if such an antibody represents a critical pathologic epitope and idotype among patients. Knowing such epitopes and idiotypes may result in the ability for more specific diagnosis, prognosis and monitoring of disease. For example, anti-Dsg enzyme-linked immunosorbent (ELISA) assays in which measurement of the degree of blocking by a pemphigus patient's serum of pathologic anti-Dsg mAb binding would be a measure of the amount of pathologic antibodies in that serum. One could also potentially target therapy to idiotypes associated with pathogenicity.

Cloning Dsg-specific pathologic antibodies will produce valuable reagents to study how antibodies cause cell-cell separation (acantholysis) in pemphigus. Finally, cloning Dsg-specific non-pathologic antibodies could be useful for targeting biologically active proteins in the epidermis.

Phage display cloning of pemphigus mAbs

Phage display is a powerful technique in which PCR is used to clone the heavy and light chain variable region of the peripheral B cells into a vector that creates a phage particle with the antibody expressed on its surface and the cDNA encoding that antibody inside (Figure 1).¹³ The antibody on the surface is expressed as a single chain variable fragment (scFv) that contains only the variable light and heavy chain that fold into the proper antigen-binding configuration. The library of phages produced from a PF or PV patient are then panned on a plate containing Dsg1 or Dsg3; or, in some cases, alternating between the two. In this way specific clones are isolated that specifically bind Dsg1, Dsg3 or both Dsg1 and Dsg3. The phage display technique is explained in more detail in Figure 1, and in very great detail by Barbas *et al.*¹³

ScFv mAbs isolated from pemphigus patients in this way can be produced in soluble form by recombinant technology in *Escherichia coli* and purified on a metal affinity column because they contain a histidine tag (H6). Such antibodies bind to Dsg (Figure 2) and bind the cell surface of keratinocytes, as does pemphigus IgG from patients (Figure 3).

Characterization of pemphigus mAbs

The soluble scFv could be used to test for pathogenicity either by injecting into normal human skin organ culture or into neonatal mice. If the antibodies are pathogenic they will cause typical pemphigus blisters in these models. An example of a pathogenic anti-Dsg1 mAb cloned from a PF patient and injected into normal human skin is shown in Figure 4.

The cDNA of each clone is sequenced to characterize the genetics of the pathogenic and non-pathogenic monoclonal antibodies. Results for such studies^{7,8} show that in both PV and PF patients the heavy chain (VH) genes used for Dsg-binding antibodies are severely restricted whereas the light chain genes (VL) are more promiscuous (i.e. different light chains can pair

with the same heavy chain). The immunochemical characteristics of the antibodies (e.g. binding to denatured or native antigen, immunofluorescence binding to human and/or mouse skin, binding to other Dsgs, pathogenicity) correlate with the VH, not the VL, gene. PV and PF patients have both pathogenic and non-pathogenic mAbs, and most of the isolated antibodies are non-pathogenic.

For example, in screening a phage display library from a PF patient, the anti-Dsg1 antibodies found used only five VH genes, but many different VL genes were used. Only two antibodies were pathogenic, using only two VH genes. The immunochemical properties of these different antibodies correlated with their VH gene usage.

The pathologic mAbs found from the PF library were blocked by most PF sera, suggesting that these mAbs define common pathologic epitopes on Dsg1, and may share structures (i.e. idiotypes) in order to bind these common epitopes.

These data show that the antibody response, especially the pathogenic response, is restricted and, therefore, it may be feasible to target the specific pathogenic antibodies for therapy.

Blocking the effects of pathogenic pemphigus mAbs with peptides isolated by peptide-phage display

To determine which reagents could block pathologic pemphigus mAbs we have used peptide-phage display (Figure 5).¹⁴ Monoclonal pathogenic antibodies were used to screen peptide libraries to find short peptides that interfere with antibody binding. In screening such a peptide library with one of the pathogenic scFv isolated from a PV patient, we found several peptides that blocked the pathogenic mAb. These peptides also blocked another pathogenic antibody with the same VH gene usage, showing that it is feasible to block pathologic antibodies by targeting just their heavy chain variable region.

Conclusions

Taken together, these data suggest that the pathogenic antibody response in pemphigus is severely restricted, at least in individual patients. As pathogenic antibodies found by phage display bind Dsg at or near epitopes bound by sera from many other pemphigus patients, it may be feasible to target shared properties of pathologic antibodies across patients to treat disease. However, the practicality of such an approach awaits cloning of antibodies from additional pemphigus patients to determine to what degree antibody properties are preserved among different patients.

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Figure 1.

Technique of phage display to clone scFv mAbs against desmoglein. (a) PCR is used to amplify the variable heavy (V_H) and light (V_L) chains from a pemphigus patient's B cells, which are then cloned into a phage vector that contains DNA encoding: a linker (L), 6 histidines ($H6$), a haemagglutinin decapeptide tag (HA), and the P_{III} coat protein of M13 filamentous phage. (b) Each individual phage displays an scFv on its coat and contains the DNA encoding that scFv. (c) The library of phages from the pemphigus patient is panned on Dsg to isolate those phages that bind Dsg; the bound phages are cloned and their DNA sequenced.

Figure 2. Single chain variable fragment (scFv) cloned from a PV patient binds Dsg3. Lane 1, Coomassie Blue stain of SDS-PAGE shows total cell extract of cultured human keratinocytes. Lane 2, immunoblot of the extract with the scFv stains the 130-kD band of Dsg3.

Figure 3.

Indirect immunofluorescence on normal human skin with scFv cloned from a PV patient. Dsg3 is on the cell surface of basal cells whereas Dsg1 is present on the cell surface of keratinocytes throughout the epidermis.

Figure 4. Anti-Dsg1 cloned from a PF patient causes a typical superficial epidermal blister when injected into normal human skin organ culture.

Figure 5.

Peptide phage display. A library of phages expressing different random peptides of 12 amino acids each is tested against a single pathogenic mAb isolated from a pemphigus patient. The mAb is bound to magnetic beads (Mag Bead) by interaction with Protein G (G) and the bound phages are isolated with a magnet. The isolated phages are cloned and their DNA sequenced to determine the peptides that bind the pathologic antibody.