

# A Pemphigus Vulgaris with IgG1 and IgG4 Subclass Autoantibodies

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The distribution of pemphigus subclass autoantibodies in a patient with pemphigus vulgaris (PV) has been investigated by semiquantitative indirect immunofluorescence (IIF), using the HP series monoclonal antibodies specific for four human IgG subclasses on human foreskins. IgG1 and IgG4 intercellular substance-specific autoantibodies were detected in the serum of the patient, whereas IgG2 and IgG3 autoantibodies were absent.

In addition to foreskins, human tonsillar epithelia were used as substrates of IIF for detecting the PV autoantibodies and it was one of satisfactory substitutes for monkey esophagus. (*Ann Dermatol* 2:(1) 35-38, 1990)

*Key Words:* Human tonsillar epithelium, Monoclonal antibodies specific for human IgG subclasses, Pemphigus subclass autoantibodies.

Pemphigus vulgaris (PV) is characterized immunologically by circulating IgG autoantibodies which react with antigens located in the intercellular substance (ICS) of the epidermis,<sup>1,2</sup> or on the surface of keratinocytes.<sup>3</sup>

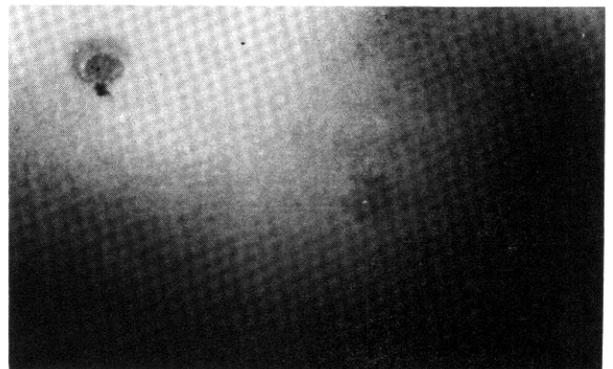
Human IgG can be subdivided into four highly homologous subclasses and differences in the biological properties of their human IgG subclasses were reported.<sup>3</sup> The investigation of the distribution of PV autoantibodies in the IgG subclasses would be of great help in understanding their pathophysiologic roles in PV. Sams and Schur<sup>4</sup> had tested PV sera from three patients using polyclonal monkey and rabbit antisera to human IgG subclasses, and ICS antibody activity was detected in the all four IgG subclasses. But, Jones et al.,<sup>5</sup> who used the monoclonal antibodies (MoAb) to human IgG subclass, could detect only IgG4 and IgG1 subclasses which are the main components of PV autoantibodies.

We present here a case of pemphigus vulgaris who had pemphigus IgG1 and IgG4 subclass autoantibodies, as detected using MoAb specific for the human IgG subclasses.

We also investigated the efficacy of human tonsillar epithelium as a substrate in IIF study of PV.

## REPORT OF A CASE

A 48-year-old man visited our hospital with several recurrent vesicles and bullae on the upper trunk for one year duration. His past history rev-



**Fig. 1.** A ruptured flaccid bulla with denuded base on the chest.

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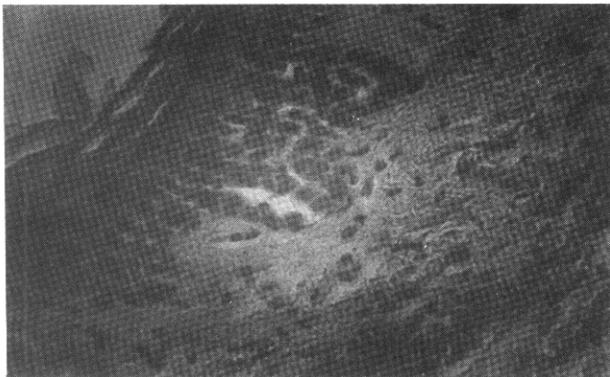
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ealed recurrent painful erosions on the oral mucosa. Family history was not contributory.

This patient had several ruptured flaccid bullae with denuded bases on his chest (Fig. 1), and several crusted vesicles on his upper back. Nikolsky's sign was detected on the peribullous lesions. There was no lymphadenopathy.

Laboratory studies, including complete blood cell count with differential, serum concentration of immunoglobulins (IgG, A, M, and E), C3 and C4 concentration, and fibrinogen were within the normal ranges.

Histologic examination of a skin specimen obtained from a vesicular lesion on the chest showed diffuse acantholysis in the lower epidermis and focal clusters of inflammatory cells in the dermis. On the high power view, suprabasal intraepidermal cleft and dermal infiltration of lymphocytes were noted (Fig. 2).



**Fig. 2.** Biopsy specimen from a vesicular lesion shows diffuse acantholysis and suprabasal intraepidermal cleft in the lower epidermis and dermal infiltrate of lymphocytes with cluster formation (H & E stain,  $\times 200$ ).

## IF Studies

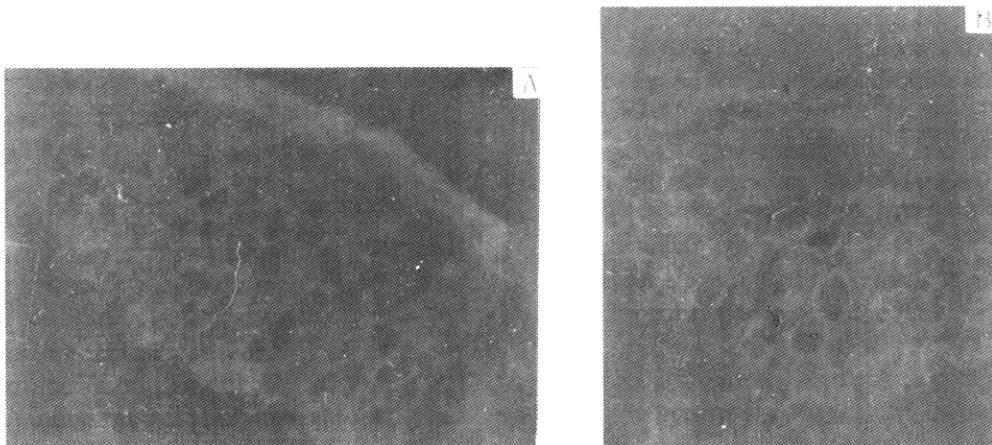
Direct immunofluorescence of the patient's perivesicular specimen demonstrated linear intercellular deposits of IgG and C3.

IIF studies on human tonsillar epithelium (obtained by tonsillectomy in patient with chronic hypertrophic tonsillitis) were performed with polyclonal antihuman IgG reagent, according to the procedure by Gligora.<sup>6</sup> A linear deposit of IgG in the ICS region was demonstrated, the same or even stronger intensity than those seen on the human foreskin substrate (Fig. 3).

To determine pemphigus IgG subclasses, first the patient's serum, then human IgG subclass specific MoAbs (HP 6002, HP 6017, HP 6025, HP 6050, HP 6069; Hybridoma Reagent Laboratory, The University of Texas Medical School at Houston) were applied to the foreskin. After washing with phosphate buffered saline solution plus 4% bovine serum albumin, the foreskin was incubated with



**Fig. 3.** Indirect immunofluorescent staining of human tonsillar epithelium with polyclonal antibody to IgG ( $\times 200$ ).



**Fig. 4.** Indirect immunofluorescent staining of human foreskin sections with monoclonal antibodies to IgG subclasses ( $\times 400$ ). IIF staining for IgG1 (A) and IgG4 (B)

FITC-conjugated goat anti-mouse IgG and examined with a fluorescence microscope.

Both IgG1 and IgG4 subclasses demonstrated continuous linear intercellular staining with delicate, smooth, apple green fluorescence (Fig. 4). The titers of IgG1 and IgG4 were the same, at 1:40, but IgG2 and IgG3 subclasses were not detected at all.

## DISCUSSION

In our PV case, the indirect IF study showed that IgG1 and IgG4 subclasses are the main components of PV autoantibodies, which concurs with the results of Jones et al.,<sup>5</sup> Rock et al.,<sup>7</sup> and Kim et al.<sup>8</sup>

In the sequence of the events that follow pemphigus antibodies binding to the pemphigus antigens on the epidermal cell membrane, there are two different but nonexclusive models. The first, pemphigus antibodies induce acantholysis through local stimulation of the plasminogen-plasmin system, an effect that occurs independently of complement.<sup>9</sup> The second is that pemphigus antibodies can fix complement, leading to acantholysis.<sup>10, 11</sup>

In order that complement can be involved in producing acantholysis, the components of PV autoantibodies should be the subclasses of IgG which can fix complement; IgG1, IgG2 and/or IgG3.

We could confirm that IgG1 is one of the main subclasses in our PV case. As we could not find any IgG2 and IgG3 antibody activities, it differs from the report by Sams and Schur<sup>4</sup> who found all four IgG subclasses using polyclonal antisera rather than the specific MoAbs. Considering the specificity of their reagents, we used MoAbs to each of the four human IgG subclasses, of which specificity and immunoreactivity was already confirmed and used in previous studies.<sup>3, 5, 12</sup>

In our study, it is remarkable that IgG4 was detected as one of the main subclasses in PV autoantibody IgG, in view to the fact that it is the least common in normal human serum, 0.7-4.2% of the total serum IgG.<sup>13, 14</sup>

Human IgG4 subclass takes a special place in the immune response of chronic exposure to antigen. For example, it is proved to inhibit immune precipitation and binding of C1q to IgG1, thus may

be considered as a protective against the biological effects of the complement-fixing antibodies, and possibly prevents an autoimmune process.<sup>15</sup> Thus, it would be an interesting study to evaluate what the roles of pemphigus autoantibody IgG subclasses are in the pathophysiology of PV.

Andersen and Hale<sup>16</sup> recommended monkey esophagus as the best sample tissue for the intercellular antibodies (IC Ab). But, there is an obvious problem of obtaining monkey esophagus for the tissue test antigen. Considering the ontogenic and phylogenic development of organs and their association with the immunological system, the epithelia of human lymphoid organs, particularly the palatal tonsil was first introduced by Gligora<sup>6</sup> who revealed that tonsillar epithelium was more sensitive to IC Ab than monkey esophagus in PV. In our study, tonsillar epithelium was a satisfactory substrate because it demonstrated bright IF on ICS. Since tonsillectomies are carried out daily, even in small hospitals, it is easier to obtain the tonsillar epithelium. Thus, tonsillar epithelium can be an adequate substitute for monkey esophagus to detect PV autoantibody IgG in serum.

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