Production of Pemphigus Antibody \textit{in Vitro} and Analysis of T-Cell Subsets

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T cells from nine patients in the active stage of pemphigus vulgaris and five in the inactive stage of the disease were studied with Leu-1, Leu-2, and Leu-3 monoclonal antibodies. No significant differences were observed in the proportions of total T cells or T cells expressing either helper or suppressor phenotype in peripheral blood leukocytes of patients compared to normal subjects. Immunoregulatory mechanisms were functionally studied using an assay measuring total IgG synthesized \textit{in vitro}. Peripheral blood leukocytes were separated into T- and B-cell fractions and cultured in various combinations. In nine experiments, the T cells were irradiated prior to culturing with B cells to remove their suppressor function. No statistically significant differences were observed in the total IgG synthesized by B cells obtained from patients and normal subjects when cultured with untreated T cells or irradiated T cells obtained from patients or normal controls. These results indicated that there was no loss of suppressor-cell function or increased helper-cell function when assessed by measuring the total IgG synthesized. The addition of serum from pemphigus patients to peripheral blood leukocyte cultures of pemphigus patients and normal controls had no statistically significant effect on the synthesis of total protein or on the amount of Ig synthesized and secreted. Peripheral blood leukocytes from six untreated patients with pemphigus vulgaris were stimulated \textit{in vitro} with pokeweed mitogen (PWM) to produce immunoglobulin. The IgG produced selectively bound to the intercellular cement substance of the epidermis of patients' perilesional skin, normal human skin, and monkey esophagus. The IgG was biosynthetically labeled by culturing the leukocytes in medium supplemented with \textsuperscript{3}H]leucine, and the binding of the radiolabeled IgG was visualized by autoradiography. The IgG nature of the protein was demonstrated by precipitation with \textit{Staphylococcus} protein A and removal with rabbit anti-human IgG antisera. Peripheral blood leukocytes obtained from normal volunteers and control patients did not produce this antibody. Our studies indicate that there was no general functional or phenotypic alteration of suppressor or helper T cells in the peripheral blood. The peripheral blood leukocytes of pemphigus patients under PWM stimulation can produce an anti-intercellular cement substance antibody \textit{in vitro}. These results indicate that the abnormality of immunoregulation which resulted in the production of a pathogenetic autoantibody in pemphigus is highly specific.

\textbf{KEY WORDS:} Pemphigus vulgaris; immunoregulation; monoclonal antibodies; autoradiography.

\textbf{INTRODUCTION}

Pemphigus vulgaris is a chronic blistering autoimmune disease of the skin and mucous membranes which is associated with severe morbidity and significant mortality (1). It is characterized histopathologically by an intraepidermal blister (bulla) with acantholysis of epidermal cells (2). The deposition of immunoglobulins and components of the alternate and classical pathway of complement in the intercellular cement substance (ICS) of the epidermis can be demonstrated by direct immunofluorescence (3). Circulating antibody directed against the intercellular cement substance of the stratified squamous epithelium of the skin and mucous membranes can be demonstrated in the serum of these patients (4). The titer of this antibody, determined by indirect immunofluorescence using monkey esophagus as substrate, correlates with the extent and severity of disease (5), and clinical improvement of the disease is associated with a decrease in
antibody titer (6). Schiltz and Michel have produced histologically and immunopathologically pemphigus-like lesions in explants from normal human skin maintained in tissue culture medium supplemented with partially purified IgG fraction of pooled sera from patients with pemphigus (7, 8). Transfusion of high-titer pemphigus serum into monkeys resulted in the deposition of the antibody in the epidermal ICS but did not produce clinical or histopathological disease (9).

The regulation of immunoglobulin synthesis of T cells is well documented (10). In murine systems both nonspecific and antigen-specific T cell-mediated help and suppression of antibody synthesis have been reported (10, 11). Similar observations have been made on T cell-mediated regulation of nonspecific and antigen-specific immunoglobulin synthesis by peripheral blood lymphocytes from humans (12).

Autoantibody formation requires a break in the immunological tolerance usually displayed toward self-antigens. A lack of T suppression is one mechanism that might underlie the breakdown of tolerance, resulting in the production of autoantibodies (13).

Studies on cellular function and regulation of specific or nonspecific Ig production in pemphigus are lacking. Pokeweed mitogen (PWM)—stimulated IgG synthesized by peripheral blood leukocytes (PBL) has been used to study the immunoregulation of both nonspecific and antigen-specific IgG synthesis by PBL from humans (14, 15), and the system has been adapted to analyze both T cell-mediated help and cell-mediated suppression of immunoglobulin synthesis (16, 17). Since the in vitro production of immunoglobulins from different individuals is not substantially affected by allogenic effects (16, 18), combinations of subpopulations derived from normal and patient lymphocytes can be utilized to study the immunoregulatory capacity of their T-cell populations. In addition, studies by several investigators have shown that T suppressor-cell function is radiosensitive while T helper-cell function is relatively radioresistant. (19, 20).

Monoclonal antibodies have been developed recently that define antigens that represent phenotypic markers for functionally distinct subsets of human T lymphocytes. Thus, they provide the opportunity to assess simultaneously various subsets of T cells in the peripheral blood. The Leu-1 antibody reacts with virtually all normal peripheral blood T cells (21), while the Leu-3 and Leu-2 antibodies define subsets of T cells that are capable of mediating helper/inducer (Th/i) and cytotoxic-suppressor (Tc/s) activity respectively (22, 23). Recent reports have indicated that imbalances in the T-cell subsets defined by these and similar monoclonal antibodies are associated with a variety of disease states, including viral-induced immunodeficiencies (24), multiple sclerosis (25), systemic lupus erythematosus (26), and juvenile rheumatoid arthritis (27).

The present study was undertaken to determine the distribution of the subsets of T cells in the peripheral blood of patients with active pemphigus. A functional assay to evaluate the T- and B-cell interaction was done simultaneously. The effect of pemphigus antibody on the production of Ig and total protein was evaluated. Studies were directed at examining the ability of PBL from pemphigus patients to produce an anti-ICS antibody in vitro, under pokeweed mitogen (PWM) stimulation. An autoradiographic assay was developed to evaluate the in vitro production of the anti-ICS antibody.

MATERIALS AND METHODS

All the patients presented in this study were seen and treated at UCLA Hospital and Clinics. Patients presented with flaccid bullae or erosions on the skin and mucous membranes. The diagnosis of pemphigus vulgaris was confirmed histologically and immunopathologically. Patients studied during the acute stage of the disease were not receiving any systemic therapy at the time of the study. Patients studied during the inactive stage of the disease were clinically free of disease and had been off all therapy for a minimum of 6 months.

Normal controls were volunteers from the UCLA community. In the experiment for the in vitro production of anti-ICS antibody, additional control subjects included two patients with immunopathologically proven bullous pemphigoid, one patient with systemic lupus erythematosus, one patient with dermatitis herpetiformis, and one patient with psoriasis.

Immunopathological Studies

The titer of pemphigus antibody was determined by indirect immunofluorescence using monkey esophagus as substrate (28). Four-micron-thick monkey esophagus sections were used in the indirect immunofluorescence assay and in the autoradiographic studies. Four-millimeter punch biopsies from the peribullous areas of the patients’ skin and