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T-Helper Cell Activation in Bullous Pemphigoid

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In 10 untreated patients suffering from acute bullous pemphigoid the number of peripheral blood T cells (CD 3), T suppressor (CD 8) and T helper cells (CD 4) as well as activation antigens (DR and Tac) bearing lymphocytes was evaluated by monoclonal antibodies. While the pan-T cell population (CD 3) and T suppressor subpopulation (CD 8) were normal, the T helper subpopulation (CD 4) and the number of DR and Tac positive lymphocytes were significantly increased in the acute stage of bullous pemphigoid when compared to the age- and sex-matched controls. Phenotypically those DR positive cells belonged to the pan-T cell population (CD 3) at 66% and to the T helper subpopulation (CD 4) at 53% respectively. Under treatment with immunosuppressants the cell counts returned to normal suggesting a T helper cell activation to be involved in the acute stage of bullous pemphigoid. *Key words: Lymphocyte subpopulations; Activation antigens.* (Received April 8, 1987.)

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Bullous pemphigoid (bP) is a blister forming skin disease of autoimmune nature characterized by the deposition of immunoglobulins and/or complement at the basement membrane zone (BMZ) and by freely circulating BMZ antibodies, mostly of the IgG class. No further deviations of the humoral immune system up to now is detected. Concerning the cellular immunity it is known, that the peripheral blood lymphocytes (PBL) of bP patients exhibit a normal distribution of T and B cells (1, 2, 3) and that neither a lack of suppressor cells nor an increase in the helper cell activity is found in functional assays (4, 5). Activated lymphocytes and their products may play an important role in the blister formation, because lymphokines with lymphocyte chemotractant (6) and lymphotoxin-like activities (7) have been detected in the blister fluid of bP. The proportion of lymphocyte subpopulations and activation antigens bearing lymphocytes are still quite unclear in bP patients. The aim of the present study was to quantify some of the defined subpopulations of PBL and to identify the phenotype of activation antigens bearing lymphocytes by monoclonal antibodies in bP patients before and under treatment with immunosuppressants.

PATIENTS

The patients were studied in the acute stage of bP (before treatment) as well as under therapy with immunosuppressants. All patients exhibited subepidermal blisters in histology and IgG and/or com-

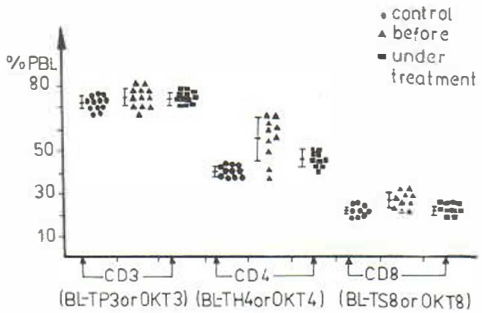


Fig. 1. T cell subsets before (▲) and under (■) therapy with immunosuppressive agents compared to controls (●).

plement deposits at the BMZ in the direct immunofluorescence method. The titres of BMZ antibodies varied between 160 and 1280. Age- and sex-matched blood donors served as control group.

METHODS

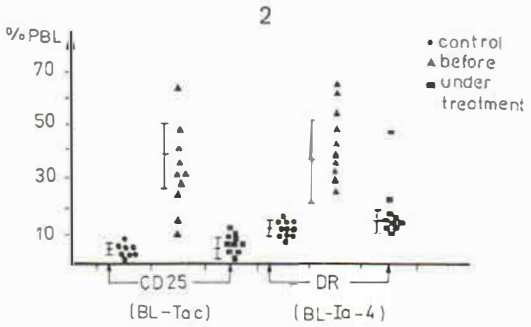
PBL were separated from peripheral venous blood by density gradient centrifugation employing the method described by Böyum (8). Cell surface antigens were determined by the slide method in the indirect immunofluorescence assay (9). 0.02 ml cell suspension were incubated for 10 min at +4°C on a Polydimethyldiallyl-ammoniumchloride containing slide. The cells were washed first with PBS, incubated with 0.02 ml diluted monoclonal antibodies (Ortho Diagnostic Syst., USA, and Dept. of Biosciences, Karl-Marx-University Leipzig, GDR) 30 min at +4°C and afterwards again washed twice with PBS. After another 30 min incubation at +4°C with a solution of fluorescein-labelled goat anti-mouse IgG (SIFIN Berlin, GDR) the cells were washed with PBS. The percentage of fluorescein positive cells in a total number of 200 counted cells was determined using a Jenamed fluorescence microscope (VEB Carl-Zeiss-Jena, GDR). For the determination of the phenotype of activated lymphocytes a double immunofluorescence assay with rhodamin (TRITC)-labelled monoclonal antibodies in combination with fluorescein (FITC)-labelled monoclonal antibodies (Dept. of Biosciences, Karl-Marx-University Leipzig, GDR) was used. For statistical analysis the Student's *t*-test was used.

RESULTS

As shown in Fig. 1 the percentage of pan-T cells (CD 3) and T suppressor cells (CD 8) was not diminished, while the proportion of T helper cells (CD 4) was about 15% higher ($p<0.01$) in the acute stage of bP when compared to the controls. The percentage of T helper cells (CD 4) returned to normal (47% vs. 41% in controls) under therapy with immunosuppressants. The number of activation antigens bearing lymphocytes (DR and Tac) was also significantly ($p<0.01$) increased (Fig. 2). BP patients expressed 37% DR-bearing and 29% Tac-bearing lymphocytes contrary to controls who exhibited 13% DR-bearing and 5% Tac-bearing lymphocytes. During treatment with immunosuppressants the difference between patients and controls was abolished. It is a well established fact that all B cells represent DR-positive lymphocytes and that activated T-lymphocytes bear DR antigens. In the acute stage of bP 66% of DR-bearing lymphocytes were revealed to be T cells (CD 3) (Fig. 3), 53% of DR-bearing lymphocytes belonged to T helper cells (CD 4) and 5% of them to T suppressor cells (CD 8). In the controls we did not find activated T cells.

DISCUSSION

In bP the number of T cells was demonstrated to be within normal ranges as determined by rosette techniques previously (1, 2, 3) and by monoclonal antibodies in this study. Even in functional assays neither an increased helper cell activity nor a lack of suppressor cell



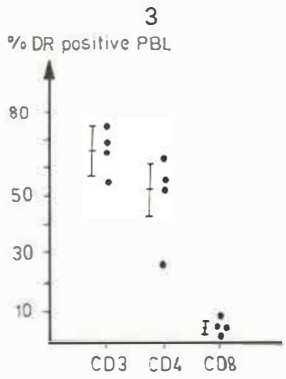


Fig. 2. Percentage of Tac and DR positive lymphocytes in acute stage of bP (▲) and under therapy (■) compared to controls (●).

Fig. 3. Phenotype characterization of activated lymphocytes (DR-positive).

activity was detected by other authors (4, 5), probably because minimal deviations cannot be recognized in these assays (4). However, in the acute stage of bP the number of T helper cells (CD 4) was significantly increased, while the number of T suppressor cells (CD 8) was not changed.

It is widely accepted that the immune response is initiated by the presentation of the processed antigen in connection with DR antigens on the cell surface of monocytic cells to T helper cells (CD 4). These cells respond with blast transformation and the expression of activation antigens such as Tac and DR. The expression of those cell surface determinants activated by T helper cells (CD 4) is accompanied by the secretion of lymphokines, which promotes the differentiation of further lymphocyte subsets (10, 11). Thus, activated lymphocytes and their products probably play an important role in the blister formation of bP (7).

Our results indicate an increased percentage of DR and Tac bearing PBL in the acute stage of bP. Using a functional assay Ahmed et al. (12) found a decreased interleukin-2 production by bP lymphocytes possibly caused by an increased proportion of Tac-bearing lymphocytes, which may consume interleukin-2. The characterization of the phenotype of activated lymphocytes showed 66% DR positive lymphocytes in the pan-T cell population (CD 3) and 53% in the T helper subpopulation (CD 4) suggesting the T helper cell activation in the acute stage of bP. Under therapy with immunosuppressants bP patients exhibited a normal distribution of lymphocyte subsets and activation antigens bearing lymphocytes. Therefore the percentage of activated T helper cells may be a marker for the disease activity in bP. The significance of activated T helper cells in autoantibody production needs further investigations.

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Carcinomas Following Grenz Ray Treatment of Benign Dermatoses

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During the years 1977–1986 five patients were referred to the Department of Plastic Surgery, University Hospital, Århus, for treatment of carcinomas occurring in the skin on the sites previously treated with Grenz rays for benign dermatoses. Grenz ray treatment had been given for eight to nineteen years. The dose given, in one case, was very extensive. Otherwise a dose between 10000 rad (100 Gy) and 29300 rad (293 Gy) was given. The tumours were squamous cell carcinomas in four cases and basal cell carcinoma in one case. One person developed both squamous and basal cell carcinomas. The tumours behaved aggressively and in one young patient the dura and the parietal bone had to be replaced by tensor fasciae latae graft and a free latissimus dorsi flap. None of the patients had been exposed to other known carcinogens. Caution in applying Grenz ray treatment is stressed. (Received February 26, 1987.)

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Grenz rays (Bucky) have been used in treating a variety of dermatoses and has been considered relatively safe with only minor adverse reactions, such as erythema, hyperpigmentation and telangiectasia (1, 2).

Grenz rays are ionizing radiation at 8 to 15 kV with a HVL of 0.018 to 0.036 mm Al. The electromagnetic waves are of relatively long wavelength (1 to 3 Å), between the borderlines of ultraviolet rays and X-rays, and were developed by Bucky in 1923 (1). The radiation effects are limited to a tissue depth of 2 to 3 mm, as thoroughly investigated by E. Ebbehøj in 1937 (3).

The first case of carcinoma caused by Grenz rays in extensive doses was reported by F. Kalz in 1959 (2). Since then 14 additional cases have been verified in the literature (4, 5, 6, 7, 8, 9).

Over a five-year-period, at the Department of Plastic Surgery, University Hospital, Århus, we have treated five patients with carcinomas following treatment with Grenz rays for benign dermatoses. Three of the patients developed metastases.