

## Bullous Lesions in Chronic Lymphocytic Leukaemia: Pemphigoid or Insect Bites?

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Sir,

Diffuse bullous lesions may occur during the course of B-cell chronic lymphocytic leukaemia (B-CLL). Some of them have been reported to show the clinical and histological features of pemphigus vulgaris (1) or paraneoplastic pemphigus (2, 3). However, other eruptions with different clinical and histological features have been described. These rashes have been diagnosed generally as a 'bullous pemphigoid-like disease' or an abnormal reaction to insect bites (4–8). The purpose of our study was to verify how many patients with B-CLL may develop this latter type of bullous eruption and which pathogenetic mechanism can explain this reaction.

### SUBJECTS AND METHODS

From June 1991 to May 2004, all patients with B-CLL and relevant skin lesions were referred from the Department of Hematology at the University of Rome "La Sapienza" to the Department of Dermatology. Subjects with bullous eruption were selected and submitted to skin biopsy for routine histopathology and direct immunofluorescence studies. During summer 2000, serum samples were also taken at the time of the eruption, stored frozen (–20°C) and sent to the University of Genoa for indirect immunofluorescence and immunoblotting tests. In the same period serum samples from a comparable group of patients with B-CLL without bullous disease were also sent to Genoa. All the indirect studies were performed at the same time.

Routine histopathological examination was performed on sections prepared from skin biopsies and stained according to standard procedure (H&E). For direct immunofluorescence, skin biopsy specimens were frozen on a cryostat chuck. Three sections 4 mm thick were placed on each slide for a total number of 10 slides. The slides were air-dried at room temperature for 15–30 min and then incubated with fluorescein-conjugated antibodies to IgG, IgA, IgM and C3 (YLEM Laboratories, Rome, Italy) for 30 min in a humidity chamber. After washing with phosphate-buffered saline (PBS), the slides were examined under a Leitz epifluorescence microscope.

For indirect immunofluorescence, normal human skin was obtained from mammoplasty with the informed consent of the patient. Epidermis was split from dermis according to the standard technique (9). Six cryostat sections of normal human skin (NHS) and salt split skin (SSS) were cut in our laboratory. All sera were diluted at 1:20 in PBS and incubated for 30 min on the sections. After washing with PBS, the slides were incubated with FITC-labelled anti-human IgG goat serum (Kallestad Diagnostic, Chaska, MN, USA) for 30 min. After a further washing with PBS, they were mounted in PBS-glycerin and examined under a fluorescent microscope.

For the immunoblotting test, epidermal proteins were obtained from normal human keratinocyte cultures using standard procedures. Epidermal proteins were separated by 6% SDS-polyacrylamide slab minigel electrophoresis under reducing conditions as described previously (10). The proteins were

electrophoretically transferred to nitrocellulose filters. Nitrocellulose strips were then sequentially developed using biotinylated goat anti-human IgG (Dako, Glostrup, Denmark) and BCIP/NBT-buffered substrate tablets (Boehringer Mannheim, Germany).

We used a commercial enzyme-linked immunosorbent serologic assay (ELISA) kit for the diagnosis of pemphigoid consisting of microwells coated with the non-collagenous domain (NC16A) of the BP180Ag (MBL, Naka-Ku Nagoya, Japan). To compare the results from different plates, the test sample optical densities were adjusted according to positive and negative control samples (PC and NC) supplied in each kit. The final results were expressed as a percentage according to the instructions supplied by the manufacturer. The cut-off value was 9.

### RESULTS

Eleven patients with bullous eruption were included in the study. They represented 1% of all B-CLL patients ( $n=1010$  patients). There were six men and five women with a median age of 63 years (range 61–67). Before the dermatological observation, they had been diagnosed with B-CLL for 3 years (range: 1 month to 12 years). According to the staging of Rai and Binet for B-CLL, two patients were in stage 0/A, four in stage I/A, two in stage II/B, two in stage III/B and one in stage III/C. Five cases were under treatment with chlorambucil and corticosteroids, while the other six were not.

As controls we enrolled 16 patients with B-CLL who did not present bullous skin eruption. The controls were eight men and eight women with comparable median age of 62 years (range 60–69). According to the staging for B-CLL six patients were in stage I/A, seven in stage II/B, two in stage III/B and one in stage III/C. Five cases were under treatment with chlorambucil and corticosteroids and four with fludarabine, while the other seven subjects were not under treatment.

On clinical examination, all cases exhibited vesicles and bullae in variable number, from less than 5 to more than 20 (Fig. 1). Vesicles and bullae often arose on erythematous or urticarial lesions and sometimes were haemorrhagic. The lesions were mostly located on the upper and lower limbs; more rarely on the face or on the trunk. Histologically, oedema of papillary dermis with subepidermal blistering was evident (Fig. 2a), while a lymphocytic infiltrate with several eosinophils was observed throughout the dermis (Fig. 2b). Immunohistochemistry revealed that lymphocytes were UCHL-1 (Ubiquitin carboxyl-terminal hydrolase isozyme L1) + and CD20-, different from leukaemic lymphocytes



Fig. 1. On clinical examination, all cases exhibited vesicles and bullae in variable numbers.

present in the peripheral blood (CD5+ CD19+ CD20+). Direct immunofluorescence disclosed deposits of IgG and complement (C3) along the dermo-epidermal junction in only one patient. Indirect immunofluorescence on NHS was negative in all cases. Using SSS as substrate only one patient showed IgG deposits both on the roof and the floor of the bulla. Using immunoblotting technique, in 6 of 11 patients with B-CLL and bullous eruption we found serum antibodies co-migrating with an antigen of 180 kDa that has the same molecular weight as the minor bullous pemphigoid antigen. Four of the control sera showed the same pattern of immunoreactivity; three of them had been treated some weeks before with fludarabine.

All patients with skin eruption were treated with prednisone at the dosage of 10–50 mg per day, depending on the stage of B-CLL and on the general clinical conditions. After 2–4 weeks of therapy all the patients presented a complete or partial response. Four of them had summer relapses in the following years. Ten patients are still alive; in one leukaemia proceeded to a fatal outcome.

## DISCUSSION

In 1971, Bureau et al. (4) reported on six B-CLL patients with a bullous eruption characterized histologically by a dermo-epidermal cleft with intra-epidermal multilocular vesicles, oedema of the papillary dermis and a dermal lymphocytic infiltrate extending to hypodermis and adnexa. They regarded these pemphigoid-like eruptions as specific lesions of B-CLL. A pemphigoid-like disease was

also proposed in 1973 by Fayolle et al. (5) reporting on seven patients with a bullous eruption observed among a group of 430 B-CLL patients. On the contrary, in 1986 Rosen et al. (6), reporting on 10 cases with a similar bullous eruption, suggested that the lesions represented an unusual reaction to arthropod bite, secondary to the underlying lymphoproliferative disorder. The same conclusion was reached by Pedersen et al. (7), describing three patients with similar features.

In all our cases except one, direct and indirect immunofluorescence studies were negative. However, most of the immunoblotting tests were positive, revealing the presence of a serum antibody directed against a 180-kDa antigen, similar to that of bullous pemphigoid. According to these data we hypothesize a possible pemphigoid-like eruption in patients with B-CLL determined by a serum antibody revealed only by immunoblotting tests. The constant presence of eosinophils inside the dermal infiltrates of our patients is in accordance with a pemphigoid-like reaction. However, we cannot completely rule out an insect bite aetiology. We would like to suggest that this bullous pemphigoid-like eruption could appear in patients with B-CLL as an abnormal immune response after insect bites but we cannot support it with any significant experimental or clinical data. As far as the prognosis is concerned, this skin eruption seems not to be related to a fatal outcome. In fact, only one of our patients died during the follow-up.

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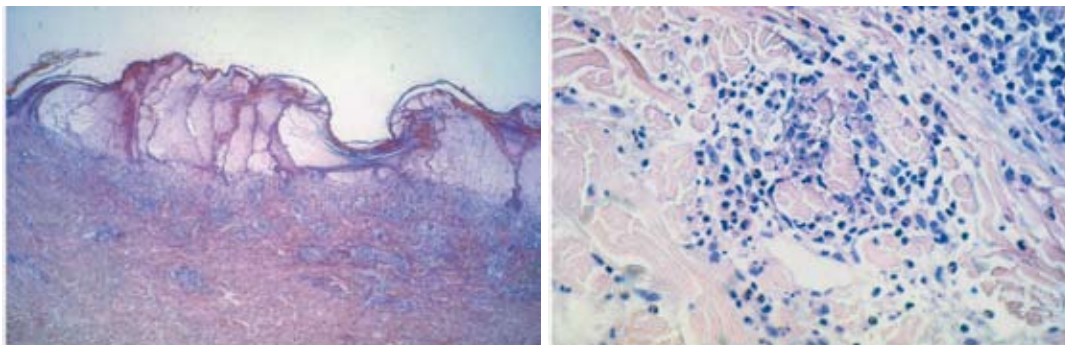


Fig. 2. (a) Histologically, oedema of papillary dermis with subepidermal blistering was evident in all the lesions (H&E,  $\times 10$ ). (b) At higher magnification, lymphocytic infiltrates with several eosinophils were often observed throughout the dermis. (H&E,  $\times 40$ ).

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