The application of microwave irradiation in immunofluorescence for the diagnosis of autoimmune bullous disease

SIR, Microwave irradiation has been applied for the fixation of specimens in electronmicroscopy and the retrieval of antigen from fixed samples.[1] In this study, we employed this technique for the diagnosis of bullous pemphigoid (BP) using a commercially available microwave oven. The staining intensity was almost comparable with the conventional method and the incubation time was reduced. This method allows the simple and quick diagnosis of autoimmune bullous diseases. Microwave fixation produced good preservation of cytomorphology and stable cytoplasmic and membrane antigens as well as decreased shrinkage. Microwave irradiation has been applied in the immunohistochemical staining of renal biopsies when the staining intensity was comparable with that of the conventional method.[2] Furthermore, microwave irradiation retrieved antigens in specimens fixed with formalin and embedded in paraffin wax. The method has been used for the immunohistochemical analysis of several antibodies, e.g. p53, Bcl-2, Ki-67, CD antigens, interleukin-1 and tumour necrosis factor.[3–5] Direct immunofluorescence of a skin biopsy is frequently used for the diagnosis of bullous diseases.[6,7] We studied five patients with the typical clinical features and immunological phenotype of BP. Specimens were taken from erythematus skin around a bullous lesion of BP. These specimens were immediately embedded in OCT compound (Miles, Elkhart, IN, U.S.A.), frozen in liquid nitrogen and stored at −80 °C. Cryostat specimens were cut and mounted on poly-L-lysine coated slides and subsequently air dried at room temperature for 20 min, followed by soaking in phosphate-buffered saline (PBS) for 10 min. The sections were then covered with 200 μL of fluorescein-isothiocyanate conjugated rabbit anti-human IgG antibody (10 μg/ml, DAKO, Glostrup, Denmark). The antibody concentration had already been optimized to reduce background staining in the dermis and obtain a maximum staining for autoimmune bullous diseases. Slides were then placed in a microwave oven (RE-M15, Sharp, Osaka, Japan) and exposed to microwaves for 0·5–6 min at 500 W. To avoid overheating of the diluted antibody, we put a beaker filled with 500 mL of water in one corner of the microwave oven. After irradiation, these slides were washed three times for 5 min with PBS. As a positive control, we used a conventional method. A slide was incubated at 37 °C for 40 min instead of using microwave irradiation. As a negative control, a slide was incubated at 37 °C for 6 min (the maximum irradiation time with microwave). Immediately after washing, the specimens were mounted and observed under fluorescence microscopy (Olympus, Tokyo, Japan).

When comparing the staining intensities of microwaved slides, the staining intensity increased with time. However, the intensity of the slide irradiated for 6 min was weaker than that irradiated for 5 min (Fig. 1). Irradiation for 5 min showed the strongest staining with results similar to those of the conventional method (incubation at 37 °C for 40 min, Fig. 2a). This intensity was strong enough to allow us to make a diagnosis of BP. According to this result, we decided that 5 min incubation with microwave was optimal for direct immunofluorescence. Similar results were obtained using the optimal incubation time for the other four patients (data not shown). As a negative control, we incubated specimens with the antibody at 37 °C for 6 min. We prepared three specimens each from five patients for both microwave irradiation and the

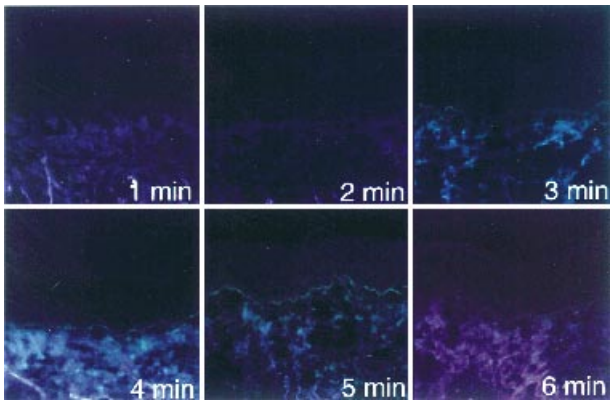

Figure 1. Direct immunofluorescence of BP after 1–6-min microwave irradiation. Five minutes of microwave irradiation shows the strongest staining intensity.

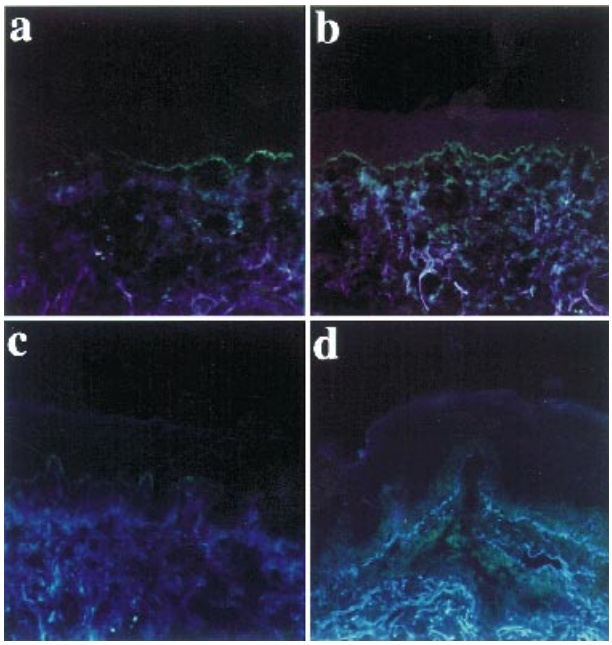

Figure 2. Comparison of pictures among the conventional method for 40 min incubation at 37 °C (a), 5-min microwave irradiation (b) and the conventional method for 6-min incubation at 37 °C as the negative control (c,d). The staining intensity with the microwave is similar to that of the conventional method. All of the specimens from three patients in the negative control showed completely negative staining (d). The specimens from the other two patients in the negative control showed discontinuous or weak linear staining, which was clearly different from those irradiated with microwave (c).

negative control. Again, we obtained strong staining in all specimens with microwave irradiation (Fig. 2b). In contrast, all of the specimens from three patients in the negative control showed no staining (Fig. 2d). However, the specimens from the other two patients in the negative control showed discontinuous or weak linear staining, which was clearly different from those irradiated with microwave (Fig. 2c).

Our study has been shown that we are able to make a diagnosis of autoimmune bullous disease using microwave irradiation. The staining intensity in the microwave-irradiated sections was comparable with that obtained by the conventional method. The incubation time in the conventional method is usually 40 min to 1 h. However, microwave irradiation required only 5 min.

In addition to the staining of known antigens it may be possible that microwave irradiation would retrieve unknown antigens. The mechanism of antigen retrieval by microwave remains unclear. Microwave energy is a non-ionizing electromagnetic wave and microwave itself increases the molecular movement of dipolar molecules such as H_2O. Microwaves generate heat rapidly and facilitate the formation of antigen–antibody complexes. However, if microwaves are too strong, it might cause overheating, resulting in the loss of antigenicity. Therefore, the time and strength of irradiation should be adjusted carefully. The disadvantage of this method is the difficulty of determining the optimal staining condition. With further modification, this method may be widely used for other immunofluorescence stainings.

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Paraneoplastic pemphigus with cutaneous and serological features of pemphigus foliaceus

Sir, Paraneoplastic pemphigus (PNP) is a distinct bullous disorder associated mostly with lymphoproliferative malignancies, with characteristic clinical presentation and immunological findings.[1,2] Of diagnostic importance is a set of antibodies[1,3] directed against a 250-kDa protein (desmoplakin I), a 210-kDa protein (desmoplakin II and envoplakin),[4] a 190-kDa protein (periplakin),[5] a 230-kDa protein [bullous pemphigoid (BP) antigen BPAg1] and a not fully characterized 170-kDa protein. It was recently demonstrated that the pemphigus vulgaris (PV) antigen is involved in PNP.[6] Using enzyme-linked immunosorbent assay (ELISA) and recombinant desmoglein (Dsg) proteins, all PNP sera tested were found positive against Dsg3 and about 60% were positive against Dsg1, the pemphigus foliaceus (PF) antigen. This indicates an important pathogenic role for these anti-Dsg autoantibodies.[6–8] The pronounced involvement of the mucosa and the cutaneous bullous lesions are similar to PV, although some cases of PNP show features similar to BP or Stevens–Johnson syndrome and, not infrequently, lichen

Figure 1. Widespread erosions of the pemphigus foliaceus type are evident on the trunk, with a pronounced Nikolsky sign.