Complement Fragment C4d and Bb Levels in Inflammatory Skin Diseases (e.g. SLE, Atopic Dermatitis, Erythroderma and Pustulosis Palmaris et Plantaris) for Assessment of Complement Activation

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Takematsu, H. and Tagami, H. Complement Fragment C4d and Bb Levels in Inflammatory Skin Diseases (e.g. SLE, Atopic Dermatitis, Erythroderma and Pustulosis Palmaris et Plantaris) for Assessment of Complement Activation. Tohoku J. Exp. Med., 1991, 163 (4), 263–268 — The complement is one of the major effector system in the process of inflammation. Complement activation has been shown to occur in inflammatory dermatoses such as systemic lupus erythematosus, atopic dermatitis, erythroderma of unknown origin, and pustulosis palmaris et plantaris by the elevated blood levels of complement fragments. To clarify the complement activation, especially the alternative pathway involvement, we have measured the concentrations of classical pathway-specific C4d and alternative pathway-derived Bb in the plasma of patients with these inflammatory disorders at a mild to exacerbated stage. Only the SLE plasma showed significantly elevated Bb levels. These results suggest that assessments of plasma C4d and Bb levels may be of value in monitoring the involvement of the complement system in patients with inflammatory dermatoses with significant complement activation.

The complement is one of the major effector system in the process of inflammation. Complement activation has been shown to occur in inflammatory dermatoses such as systemic lupus erythematosus (SLE), atopic dermatitis, erythroderma of unknown origin, and pustulosis palmaris et plantaris (PPP) by the elevated blood levels of complement fragments (Terui et al. 1987). While we could surmise an activation of the classical pathway from the elevated levels of classical pathway-specific C4a, we could not estimate the extent of the alternative pathway activation in these dermatoses.

The extents of ongoing complement-activating processes have also been shown to be reflected in the concentrations of classical pathway-derived C4d

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(Milgrom et al. 1980; Nitsche et al. 1981) and alternative pathway-derived Ba (Perrin et al. 1975; Senaldi et al. 1987; Kolb et al. 1989) and Bb (Kolb et al. 1989). We found that enzyme immunoassays (EIAs) for C4d and Bb were useful in assessing the activation of the complement system in vitro (Takematsu and Tagami 1989) and in psoriatic lesional skin (Takematsu and Tagami 1990). In the present study, we have measured concentrations of C4d and Bb in the plasma of patients with the above mentioned inflammatory skin diseases as well as vasculitis and autosensitization dermatitis in order to clarify the complement activation, especially the alternative pathway involvement in these dermatoses.

**MATERIALS AND METHODS**

**Subjects**

Blood was obtained from 64 patients with the following inflammatory dermatoses: 4 with SLE, 10 with atopic dermatitis, 14 with PPP, 10 with urticarial vasculitis or leukocytoclastic vasculitis, 7 with autosensitization dermatitis, and 19 with erythroderma of unknown origin at a mild to exacerbated stage. None of the patients had received systemic or local steroid therapy, or therapy with UV light 4 weeks prior to blood collection. In 12 patients, blood was also obtained at remission after successful treatments. Thirty-one healthy adult volunteers (aged between 24 and 60, mean 45) with no inflammatory skin diseases served as normal controls. Blood was collected in 10 mM ethylenediaminetetraacetic acid (EDTA) containing a protease inhibitor nafamostat mesilate (Torii Pharmaceutical Co., Tokyo) to avoid in vitro activation of the complement system (Takematsu and Tagami 1989). The plasma was separated by centrifugation and immediately frozen at −70°C.

**C4d and Bb measurements**

Levels of C4d and Bb were measured using C4d and Bb fragment EIA kits (Cytotech, San Diego, CA, USA) (Takematsu and Tagami 1989).

**Statistics**

Means and standard deviations were calculated and were analyzed statistically by Wilcoxon rank-sum test.

**RESULTS**

**Effect of nafamostat mesilate on C4d and Bb levels**

When nafamostat mesilate was added on collection of blood, there was no significant changes in Bb levels up to 100 µg/ml of nafamostat mesilate (Fig. 1). On the other hand, spontaneous cleavage of C4 to C4d was decreased at higher concentrations of the chemical, reaching a plateau at 20 µg/ml of nafamostat mesilate. Therefore, to avoid in vitro cleavage of C4 into C4d, blood was collected in 10 mM EDTA and 50 µg/ml nafamostat mesilate.

**C4d and Bb levels in inflammatory dermatoses**

In the inflammatory disease groups which consisted of at least 7 patients, no significant increases were found in the plasma C4d or Bb levels (Fig. 2). The Bb levels in the SLE group consisting of 4 patients were significantly higher than
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When the levels of the complement split products were compared before and after successful treatments in the plasma of patients consisting of 3 patients with atopic dermatitis, 3 with PPP, 1 with vasculitis, 2 with autosensitization dermatitis, and 3 with erythroderma, there were an increase or decrease of the levels of C4d and Bb in each samples but no significant changes (Fig. 3).

**DISCUSSION**

The present study was designed to extend our previous investigation on complement activation, especially the involvement of the alternative pathway, in inflammatory skin diseases (Terui et al. 1987). We found that, when nafamostat
mesilate was added at high concentrations on collection of blood, spontaneous cleavage of native C4 into C4d was greatly inhibited. We found a significant increase of Bb levels in the SLE group consisting of 4 patients, and an exceedingly high C4d level in one SLE patient. These results seem to be consistent with the report of the elevated C3d and Ba levels in SLE patients (Perrin et al. 1975).

In the plasma of other inflammatory skin disease groups, however, we did not find any significant increases in the C4d or Bb levels, which are consistent with the failure in finding significant increases of C3a in the plasma of atopic dermatitis (Kapp et al. 1985) and that of C4d and Bb levels in psoriatic plasma (Takematsu and Tagami 1989). The normal complement fragment blood levels in most of these dermatoses suggest that the amount of complement fragments produced only in the skin lesion may not be large enough to cause their significant increases in the circulation or there may be a high turnover rate of complement fragments in certain dermatoses (Terui et al. 1987). Another possibility is the presence of a variety of controlling proteins such as C4 binding protein (Fujita and Tamura 1983), complement receptor 1 (Ross and Medof 1985; Medof and Nussenzweig 1984), factor I (Fujita and Tamura 1983), and decay-accelerating factor (Nicholson-Weller et al. 1982; Fujita et al. 1987) in the generation of C4d and Bb, or further degradation of C4d and Bb by protease released from inflammatory cells and epidermal keratinocytes, thus escaping the detection in the assays (Takematsu and Tagami 1989, 1990). Thus the plasma C4d and Bb levels may not solely be determined by respective preceding classical and alternative pathway activation.

In summary, we have shown that assessments of plasma C4d and Bb levels may be of value in monitoring the involvement of the complement system in
patients with inflammatory dermatoses with significant complement activation. Although we failed to show that the alternative pathway of complement is also activated in the inflammatory skin diseases using EIA for Bb, an assessment of the alternative pathway activation by measuring Ba levels (Perrin et al. 1975) warrants further study.

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