Increased Plasma Concentrations of Complement Modulating Proteins (C1 Inhibitor, C4-Binding Protein, Factor H and Factor I) in Psoriasis

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— By using single radial immunodiffusion we measured the plasma levels of four complement modulating proteins, i.e., C1 inhibitor, C4-binding protein, factor H and factor I in 19 psoriatic patients in comparison with those of healthy controls. Except for C1 inhibitor which was only marginally elevated, they were found to be significantly increased in psoriatic patients. When psoriatic patients were classified into subgroups based on the clinical severity, the levels of factor H and those of factor I showed a close positive correlation with the activity and extent of the skin lesions, whereas such clear relationship could not be found with C1 inactivator or with C4-binding protein. These results offer additional support for the hypothesis that the complement system is involved in psoriasis. — C1 inhibitor; C4-binding protein; factor H; factor I

In the study of the role of the complement system in inflammatory skin lesions, our attention tends to focus on the factors involved in the production of proinflammatory factors such as anaphylatoxins and membrane attack complex or those up-regulators of the complement system such as properdin. The involvement of the complement system in pathogenesis of the inflammatory changes in psoriasis has also been suggested from the demonstration of various complement components in the skin lesions (Tagami and Ofuji 1977; Beutner et al. 1978; Kaneko et al. 1980; Takematsu et al. 1986) and changes of their blood levels (Kikindjanin and Milakov 1976; Sedgwick et al. 1981; Marley et al. 1982; Ohkohchi et al. 1985). However, we cannot disregard the participation of factors that down regulate complement activation, i.e., C1 inhibitor (C1 INH), C4-binding protein (C4bp), β 1H (factor H), C3b inactivator (factor I), anaphylatoxin

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inactivator (serum carboxypeptidase N) and cell membrane glycoproteins such as decay acceleration factors, CR1 and S protein. Only in regard to C1 INH, Kapp et al. (1985) recently demonstrated a significant increase in its blood level associated with other complement components in psoriatic patients, but they made no specific elaboration on this particular finding. Moreover, nothing is still known about the behavior of other complement controlling proteins in psoriatic patients.

The aim of the present study is to assess the blood levels of the following four complement down-regulating proteins in psoriatic patients in comparison with those of normal control subjects. C1 INH not only controls C1 already in its activated form but also regulates the activation of C1 (Ziccardi 1982). C4bp is a glycoprotein with a specific binding affinity for the activated form of the fourth complement component (C4b). When C4b is bound to C4bp, it becomes vulnerable to proteolytic degradation by the enzyme, factor I (Fujita et al. 1978). Thus, the main function of C4bp is to inhibit the activity of the C3 convertase of the classical pathway C4b2a (Gigli et al. 1979). Factor H potentiates the inactivation of C3b by factor I but it also has two other important regulatory actions, i.e., factor H possesses a direct C3b inhibitory activity of its own, distinct from factor I, and it directly inhibits the activity of the alternative pathway convertases C3bBb and PC3bBb formed by the interactions of C3b, properdin and factor B in the presence of factor D (Weiler et al. 1976; Whaley and Ruddy 1976). Factor I cleaves and inactivates cell-bound C3b or C3b in solution and free C4b. Its proteolytic activities require accelerating cofactors, factor H and C4bp (Nagasawa and Stroud 1977; Pangburn et al. 1977). By cleaving C3b it prevents the positive feed back mechanism of the complement system.

MATERIALS AND METHODS

Subjects. We studied 19 patients (14 males and 5 females) with psoriasis vulgaris of various grades of severity, one male patient of psoriatic erythroderma and 2 females with pustular psoriasis. Their ages ranged from 20 to 82 years with a mean of 51 years. They were classified according to the criteria of Obalek et al. (1977) based on the activity of the disease as judged by the presence of newly developing lesions and the extent of skin lesions.

Ten healthy volunteers consisting of 3 males and 7 females aged between 5 and 68 years with no history of psoriasis or other chronic skin diseases served as normal healthy controls.

Plasma samples. Fresh plasma were collected and stored with EDTA (at a final concentration of 0.01 M) at -70°C until use to avoid spontaneous inactivation of complement.

Antisera and immunoassay. Anti-C4bp goat serum was a gift from Dr. Teizo Fujita (Associate Professor, Department of Immunoserology, Tsukuba University School of Medicine, Ibaragi). Anti-factor H goat serum, anti-factor I goat serum, and anti-C1 INH goat serum were purchased from Miles Laboratories, Inc. (Erkhardt, IN, USA). Plasma levels of complement modulating proteins were measured by single radial immunodiffusion technique according to Mancini et al. (1965). The diffusion areas with plasma samples from control subjects were measured and the plasma levels in psoriatic patients were expressed as a percentage of the mean area of normal controls; the mean areas $\pm$ standard deviations (mm^2) for C1 INH, C4bp, factor H and factor I in the control subjects were 72 ± 9 , 15 ± 2 , 25 ± 5 and $19 \pm 3 \text{ mm}^2$, respectively. (Since pure standard reference samples for these compo-

nents were unavailable, they could not be compared on the basis of their calculated absolute concentrations).

Statistics. Data were analyzed with Student's *t*-test and/or with Wilcoxon's rank-sum test and statistical significance was defined by a *p* value of less than 0.05.

RESULTS

All the plasma complement inhibitory factors studied were increased in psoriatic patients as shown in Fig. 1.

Although plasma levels of C1 INH were higher than the mean control level in 17 of 21 psoriatic patients (80%), these increases were only marginal ($p < 0.1$ with Student's *t*-test; not statistically significant with Wilcoxon's rank-sum test) and they did not clearly correlated either with the disease activity or with the extent of the skin lesions. In contrast, C4bp showed a significant increase ($p < 0.05$) but again no clear correlation was found either with disease activity or with the extent of the skin lesions.

On the other hand, levels of factor H were significantly elevated in psoriatic patients ($p < 0.05$) and they showed a good correlation with the activity of the disease as shown in Fig. 2. Furthermore, they correlated with the extent of the skin lesions as well. Factor I levels were more prominently increased in patients with psoriasis ($p < 0.01$). It also showed a clear correlation both with the disease activity and with the extent of the skin lesions (Fig. 3).

When these plasma levels of complement inhibitory factors in psoriatic patients were pooled, there was a close correlation between each other. Thus, a significant positive correlation was found between factor H and factor I ($r = 0.55$;

Fig. 1. Levels of complement inhibitors in psoriasis. All values are expressed as percentages of the mean of normal controls. Bars represent mean $\pm$ S.D. of normal controls. NS, not significant difference. * $p < 0.05$, ** $p < 0.01$ against the mean values of normal controls.

Fig. 2. Relationship of factor H levels to disease activity and to extent of skin changes. Bars represent the mean values. Otherwise, the same as in Fig. 1.

Fig. 3. Relationship of factor I levels to disease activity and to extent of skin changes. Bars represent the mean values. Otherwise, the same as in Fig. 1.

$p < 0.01$). They also showed a significant positive correlation with C1 INH and C4bp ($p < 0.01$).

DISCUSSION

The present study demonstrated that the plasma concentrations of the four modulating proteins of the complement system, i.e., C1 INH, C4bp, factor H and factor I, that down regulate complement activation are increased in the blood of psoriatic patients as compared with those of non-psoriatic individuals. Since purified standard preparations of the tested proteins were not available at the time

of our study, we could not perform quantitative determination ; we evaluated the obtained data from the patients only in comparison with the corresponding mean values of normal controls.

The increased plasma levels of complement modulating proteins in psoriatic patients were particularly remarkable in those involved in the down regulation of the alternative pathway. In comparison with the slight to moderate increases in C1 INH and C4bp, the plasma levels of factor H and factor I were markedly elevated and they showed a good correlation with the disease activity as well as with the extent of the skin involvement. In their study of various serum complement components Marley et al. (1982) encountered unexplained findings in psoriatic patients, i.e., there was decreased consumption of C3-C9 after incubation of the patient's serum with activators of the alternative pathway with zymosan or cobra venom factor despite normal levels of C3 and B in most cases. Since factor H and factor I are involved in down regulation of the alternative pathway as pivotal modulators of the convertase, it is quite plausible that the increased levels of these factors in psoriatic patients as demonstrated in the present study are responsible for the reported prevention of alternative pathway activation in psoriatic patients.

We found only a marginal increase in C1 INH level in the blood of psoriatic patients in contrast to the finding of Kapp et al. (1985). They noted its significant increase in psoriatic patients and in patients with atopic dermatitis. Furthermore, we have failed to demonstrate significant correlation of the skin lesions and the levels of C1 INH or C4bp in the present study. However, we might be able to obtain more definite positive relationship between them if we perform the measurements in much larger numbers of patients, because we have found close correlation between the level of each complement control protein. Although we have demonstrated increased blood levels of the above-mentioned four complement modulating factors, we still do not have any data to define whether their increases are only specific for psoriasis or common to inflammatory events involving the skin. Elucidation of the precise mechanisms underlying the increased levels of these complement inhibitory factors as well as the mode of action by which they exert their effect on the inflammatory changes noted in psoriasis awaits further investigation. Furthermore, we still do not have any data on cell membrane glycoproteins in psoriasis, which also regulate the classical (decay accelerating factor) (Kinoshita et al. 1985) and alternative pathways (CR1) (Medof and Nussenzweig 1984) as well as the insertion of the membrane attacking complex into cell membranes (S protein) (Dahlback and Podack 1985) ; they might play a far more crucial role in the modulation of complement activation. However, we think that at least our findings offer additional support for the hypothesis that the complement system is involved in the pathogenesis of the inflammatory changes in psoriasis.

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