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Polymorphonuclear Leukocyte Chemotaxis in Generalized Pustular Psoriasis

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We studied polymorphonuclear leukocyte (PMNL) chemotaxis in 3 patients with generalized pustular psoriasis using 3 chemoattractants: Zymosan-activated serum from both the patients and normal individuals, and n-formyl-methionyl-leucyl-phenylalanine (FMLP). These attractants were assayed against both patient-derived and normal PMNLs. All patients had quiescent skin disease at the time of assay. We found that all patients had decreased PMNL chemotaxis to autologous Zymosan-activated serum, but not to FMLP. PMNLs from normal individuals responded normally to serum from the patients. We conclude that patients with generalized pustular psoriasis, when free of symptoms, may have an isolated PMNL chemotactic defect that is restricted to serum-derived attractants. (Received August 13, 1986.)

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Generalized pustular psoriasis (von Zumbusch) is a distinctive, uncommon inflammatory dermatosis that occurs in some psoriatic patients and is characterized by an acute pustular eruption with systemic symptoms. The primary lesions are sterile collections of polymorphonuclear leukocytes (PMNLs), which may coalesce to form large lakes of pus. There are several potential causes of this enhanced exocytosis of PMNLs: local elaboration of chemotactic factors; systemic elaboration of these factors, but with localized deposition of PMNLs; or dysfunction of PMNLs. Several investigators have evaluated various aspects of PMNL function in psoriatic patients; however, the recorded results are disparate, making interpretation difficult. These studies of PMNL function can be summarized as follows: 1) increased (1–6), normal (7–9), or variable (10, 11) chemotactic activity of PMNLs from psoriatic patients; 2) local deposition or formation of chemotactic factors within psoriatic plaques and scales (12–17); 3) abnormal chemotactic activity of psoriatic sera (1, 7). Owing both to the possible dependence upon the activity and extent of the disease at the time of assay, and to the chemotaxis assay used, no clear conclusions can be drawn concerning the mechanisms of either the PMNL-derived infiltration seen in psoriasis vulgaris, or the more dramatic PMNL exocytosis seen in pustular psoriasis.

We studied PMNL chemotaxis in patients with generalized pustular psoriasis using isolated PMNLs from both patients and normal individuals, and chemotaxis was measured in response to sera both from patients and normal persons. This enabled us to separate contributions made by various components of the neutrophilic response. In addition, we used a chemoattractant not derived from serum to separate some of the humoral and cellular attributes of PMNL function.

MATERIALS AND METHODS

Isolation of PMNLs

Three healthy male volunteers ranging in age from 25–32 and three patients with generalized pustular psoriasis were selected for the study. Blood was drawn in sterile, disposable syringes and transferred im-

mediately into sterile 10 ml Veno-jet containers (Terumo Medical Corporation, Elkton, MD) containing 5–10 units of sodium heparin per ml of blood. All subsequent tubes and pipets were plastic. Three parts whole blood was then gently mixed with 1 part Histopaque (Sigma, St. Louis, MO.) and the erythrocytes allowed to sediment by graivty at 25°C for 45 min. Purified PMNLs were obtained from the leukocyte-rich plasma by double Ficoll-Hypaque density gradient centrifugation as follows: Ficoll 400 (Pharmacia Fine Chemicals, Piscataway, NJ) was adjusted to a density of 1.07 g/cc in water; this was then layered on 50% Hypaque (Winthrop-Breon Laboratories, NY, NY); the leukocyte rich plasma was next layered on top of the Ficoll-Hypaque and the tubes spun for 30 minutes at 250×g. The purified PMNLs were then harvested from the interface between the Ficoll and Hypaque. The cells were resuspended in Hank's balanced salt solution (HBSS-Grand Island Biological Company, Grand Island, NY), made 0.1% gelatin (type IV, Sigma-GHBSS). The PMNLs were then washed twice in GHBSS at 200×g and resuspended in GHBSS to a concentration of 5×10^6 PMNLs/ml. The suspensions were greater than 98% PMNLs, and cells were assayed for chemotactic activity within 3 hours.

Preparation of sera

Blood was drawn from healthy volunteers and patients in sterile disposable syringes, immediately transferred into sterile 15 ml Veno-jet containers, and allowed to clot at 25° for 30 min. Zymosan suspension (Sigma Lot 7714, 0.05 g/ml in 0.145 M NaCl/0.05 M Na_2 HPO_4/pH 7.40) was added to the serum sample at a concentration of 1 part Zymosan to 3 parts serum, and incubated at 37° for 60 min with mixing every 15 min. This Zymosan-activated serum was then spun in a microcentrifuge and the resultant supernatant used in chemotaxis assays.

Neutrophil chemotaxis assay

The under-agarose technique was used in these studies as described by Nelson (18) with the exception that 0.5% (w/v) gelatin powder (type IV-Sigma) in HBSS was substituted for heat-inactivated human serum in the agarose mixture. Neutrophil chemotaxis was measured at the end of a four-hour incubation in a 95% air—5% CO_2 tissue culture incubator and the chemotactic index determined as described by Nelson (18). Each patient was assayed in parallel to a control individual. Chemoattractants used were serial 2-fold dilutions of the sera described above and *n*-formyl-methionyl-leucyl-phenylalanine (FMLP-Sigma) at 1×10^{-8} M in HBSS. All samples were assayed in duplicate, and the chemotactic index for each expressed as a mean of the duplicates.

Case summaries

Case 1 was a 20-year-old female college student who developed generalized infantile eczema at 6 weeks of age. At the age of 5 she had the first of many outbreaks of generalized pustular psoriasis. She was first seen at the Mayo Clinic at age 8, and the diagnosis of generalized pustular psoriasis confirmed by skin biopsy. Her exacerbations have occurred approximately 1–2 times per year from that time to her last evaluation at age 17. Many of her relapses appeared to be precipitated by bacterial infections, such as group A streptococcal pharyngitis, and were associated with systemic symptoms. She is completely free of skin disease between outbreaks. There is no family history of psoriasis or other dermatologic disorder. At the time of the study, the patient's last episode of generalized pustular psoriasis had occurred 16 months previously. She was otherwise in good health, free of all skin lesions, without any sign of infection, and taking no medications.

Case 2 was a 31-year-old male who was noted at birth to have an exfoliating erythrodermic rash located primarily on his abdomen. This was persistent until age 5. He was hospitalized at the Mayo Clinic at age 18 for generalized erythroderma studded with multiple small pustules and associated with systemic symptoms. Clinical examination and skin biopsy confirmed the diagnosis of generalized pustular psoriasis. His family history revealed that his father had psoriasis vulgaris and four siblings were atopic. At the time of this study, his last generalized pustular psoriasis episode had been 12 years previously, although he continued to have exfoliative erythroderma localized to his lower extremities. He was on no medications and had no evidence of infection.

Case 3 was a 60-year-old white male with a 42-year history of psoriasis vulgaris involving his arms, shoulder, buttocks, and lower legs. At the age of 54 (36 years after the onset of his psoriasis vulgaris) he developed idiopathic membranous glomerulonephritis and was begun on 20 mg of prednisone daily. Three months later, during tapering of his systemic steroids, the patient developed generalized pustular psoriasis that resolved with topical therapy. Since that time the patient has had recurrent pustular eruptions of his lower extremities. He has one sister with psoriasis vulgaris. At the time of this study, the patient had had a pustular flare 20 months previously. He had no signs of infection and his only medication was nadolol for treatment of his hypertension.

RESULTS

The results of the chemotaxis assays are shown in Table I. Patient PMNLs were assayed with parallel control cells against patient and control Zymosan-activated serum, as well as FMLP. The chemotactic index (ratio of directed to non-directed migration) was measured as described by Nelson (18). In this system a ratio of 1.0 represents no directed migration. We have previously assayed 100 normal patients using this system (19), and found a 95% confidence interval for chemotactic index of 1.55–2.75 for Zymosan-activated serum. Therefore values of less than 1.55 represent two standard deviations below the mean; such values are shown as absent chemotaxis in Table I. Chemokinesis, or random neutrophil movement, is not reflected in the chemotactic index, since this value is a ratio. However, random PMNL movement of all patients and controls in this study was normal for our laboratory.

From Table I, it can be seen that PMNLs from all patients showed absent chemotactic response to their own Zymosan-activated serum. In addition, patients 1 and 2 showed no response to normal serum, whereas patient 3 responded to normal serum. Control PMNLs responded normally both to autologous and patient-derived Zymosan-activated serum. The results with FMLP-directed migration show that all patients and controls responded with normal or, for patients 1 and 2, enhanced chemotaxis.

DISCUSSION

The migration of neutrophils from dermal capillaries into the epidermis is an important feature of psoriasis (20). Since the movement of neutrophils out of the vascular compartment is random in the absence of an effective chemotactic signal (21), studying neutrophil function in psoriatic patients may help to elucidate the histopathologic process of this disease. In this report, we studied PMNLs from patients with a history of generalized pustular psoriasis, but who had inactive disease at the time of the study. Using autologous Zymosan-activated serum as a chemoattractant source, an absence of chemotactic activity was noted for all patients. In one patient, this inactivity was normalized when his cells were assayed using con-

Table I. *Chemotactic response of patients and parallel control individuals*[a]

	Patient serum[b]	Chemoattractant control serum[c]	FMLP[d]
Case 1			
Patient cells[e]	−	−	++
Control cells[f]	+	+	+
Case 2			
Patient cells	−	−	++
Control cells	+	+	+
Case 3			
Patient cells	−	+	+
Control cells	+	+	+

[a] Assay performed as described in the text. In our laboratory the range for chemotactic index is 1.55–2.75 (95% confidence interval) for 100 previously assayed normal individuals. Values less than this normal range for all serum dilutions are indicated by (−) for absent chemotaxis, and values greater than this range are indicated by (++) for increased chemotaxis. One case and matching control were assayed per day.

[b] Zymosan-activated serum from the patient.

[c] Zymosan-activated serum from the parallel control individual.

[d] 1×10^{-8} M FMLP in HBSS.

[e] PMNLs isolated from the patient.

[f] PMNLs isolated from the parallel control individual.

trol serum as a chemoattractant source. Control cells showed normal chemotactic activity when assayed against psoriatic sera. There was no decrease in chemotaxis to FMLP. Our patient sample is small; however, taken together, our findings suggest that peripheral PMNLs from patients with pustular psoriasis, when their disease is quiescent, may have a selective, in vitro chemotactic defect for serum-derived attractants.

A number of substances have been implicated as relevant PMNL attractants in psoriasis. These include components of complement (12–17), eicosenoids, particularly leukotriene B_4 (17, 22, 24), oxidation metabolites (25), bacterial-derived chemotactic factors (17), and epidermal thymocyte activating factor (26). All of these mediators have been isolated from psoriatic plaques and scale. In addition, the deposition of immunoglobulins within the stratum corneum implies that circulating chemotactic factors could be preferentially deposited in skin (17).

Enhanced chemotactic activity in psoriasis has been reported by several investigators. Wahba reported increased chemotactic activity of some psoriatic plasma for normal PMNLs using the Boyden chamber technique (3, 7). He also reported enhanced chemotactic response of psoriatic neutrophils to other chemoattractants. However, this finding did not correlate with the disease severity. In a study by Kwohl (11), also using the Boyden chamber technique, PMNLs from patients with psoriasis showed enhanced chemotaxis towards both psoriatic and normal sera, whereas normal PMNLs incubated with psoriatic serum displayed no enhancement of chemotaxis. The disease activity was not described in Kwohl's study, and the patients apparently did not have the pustular form of the disease. Such information is crucial for comparisons between various studies. Other investigators have shown increased chemotactic activity of psoriatic PMNLs when incubated with known chemoattractants (1–6). Enhanced activity was shown in one study to correlate directly with disease activity (27). However, decreased or variable neutrophil chemotaxis appeared when psoriatic lesions became extensive (11, 28). This latter observation has been previously explained by the presence of circulating (29) or localized (30) inhibitors of PMNL function. In our studies, patients' sera did not inhibit chemotaxis of normal PMNLs. In two of the three patients, psoriatic PMNLs had an augmented chemotactic response toward FMLP. FMLP is a chemotactic tripeptide whose receptor is shared with bacterial chemoattractants, but is distinct from the receptor for $C5_a$ (31), a major chemoattractant in serum.

In summary, we have shown in three patients with pustular psoriasis a chemotactic defect in their circulating neutrophils when the patients were free of symptoms. The defect was restricted to serum-derived chemoattractants, and appeared to reside in the PMNLs, since normal serum did not restore activity in two of the patients, and patients' sera did not inhibit activity of normal PMNLs. In contrast, response to the chemoattractant FMLP was enhanced in two patients. Since relapses of pustular psoriasis are manifested by a dramatic migration of PMNLs into the skin, it would be of interest to study the patients during such an event, since the appearance of skin lesions might correlate with recovery of the PMNL chemotactic response to serum-derived attractants.

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