Psoriatic Sera Decrease Responses of Stimulated Granulocytes from Normal and Psoriatic Subjects*

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Previous studies have demonstrated alterations in polymorphonuclear leukocyte (PMNL) function in patients with psoriasis, but results have been variable. In this study we attempted to determine whether functional changes in PMNL from psoriatics represented an intrinsic cellular defect or a response to factors in serum. We evaluated the effect of the continuous presence of autologous and heterologous serum on lysozyme release and superoxide anion (O$_2^-$) generation by psoriatic and normal PMNL, exposed to a soluble or particulate phagocytic stimulus. There were no differences in O$_2^-$ generation or lysozyme release between normal and psoriatic PMNL without serum. However, in the presence of 10% autologous serum, these responses were significantly decreased for psoriatic PMNL (p < .001). The results were not time-dependent and did not correlate with the extent of psoriatic involvement. The data support the hypothesis that serum factors exist in patients with psoriasis that may affect PMNL functions. The presence or absence of such factors could explain, in part, the differences between the various investigations of PMNL function in psoriasis.

Several studies have demonstrated alterations in granulocyte function in patients with psoriasis. These changes include enhancement of granulocyte adherence [1], random migration [2], chemotaxis [3], phagocytosis [3], antibody-dependent cell-mediated cytotoxicity [4], and hexose monophosphate shunt activity [5]. Since these alterations are not always correlated with activity or extent of disease, it is not clear that they are secondary to epidermal hyperproliferation. Moreover, it is not possible to assign an etiologic role to such findings since while polymorphonuclear leukocytes (PMNL) may be present in dermal and epidermal inflammatory infiltrates of psoriatic lesions, mononuclear cells usually predominate, and clinical pustulation is exceptional.

In the present study, we attempted to further characterize the functional changes in PMNL from psoriatics and to determine whether or not they represent an intrinsic cellular defect(s) or a response to soluble circulating factor(s) in serum. Thus we measured the effect of the continuous presence of autologous and heterologous serum on the responses of psoriatic and normal PMNLs to a soluble and particulate phagocytic stimulus, phorbol myristate acetate (PMA) and opsonized zymosan (Z), respectively. The following parameters were evaluated: superoxide anion (O$_2^-$) generation, percent lysozyme release, and the rate of phagocytosis.

MATERIALS AND METHODS

Subjects

Patients seen at the Ohio State University Hospital Dermatology Clinic with psoriasis diagnosed by established clinical and histopathologic criteria were asked to participate. Healthy laboratory personnel with no personal or immediate family history of psoriasis served as paired controls. Informed consent was obtained from all subjects. No subject was receiving systemic medications, but some psoriatics were using topical medications (corticosteroids, coal tar products, anthralin).

Preparation of Cell Suspensions and Serum

Fifty milliliters of heparinized (10 U/ml) venous blood was obtained from subjects by venipuncture. Purified preparations of PMNL were isolated by means of Hypaque-Ficoll gradients followed by dextran sedimentation and hypotonic lysis of erythrocytes. This method yielded 100–200 × 10$^6$ PMNL depending upon the peripheral leucocyte count. Viability determined by trypan blue exclusion was 98 ± 2%. The cells were suspended in a buffered salt solution consisting of 138 mM NaCl, 2.7 mM KCl, 8.1 mM Na$_2$HPO$_4$, 1.5 mM KH$_2$PO$_4$, 1.0 mM MgCl$_2$, and 0.6 mM CaCl$_2$, pH 7.4 (hereafter referred to as PiCM).

Whole blood was withdrawn simultaneously and allowed to clot at room temperature for 30 min. Serum was separated by centrifugation at 3000 rpm for 15 min and used the same day.

PMNL from psoriatic donors were always studied with a paired normal control donor. Autologous serum refers to the donor’s serum on the day of the experiment.

Preparation of Phagocytic Stimuli

Zymosan A (Sigma) was opsonized by incubating with fresh serum (5 mg/ml) from a different normal donor for 15 min at 37°C, vortexing for 5 min, centrifuging, and restoring the original concentration in PiCM. PMA (Consolidated Midland Corp., Brewster, New York) was dissolved in dimethyl sulfoxide and used at a final concentration of 0.2 µg/ml.

Superoxide Anion Production

Washed PMNL (1 × 10$^6$) in 0.8 ml PiCM were preincubated with serum (final concentration 10%) for 15 min at 37°C in sterile plastic test tubes. Cytochrome C, horse heart, type III solution (Sigma Chemical Co., St. Louis, Missouri), 0.9 mg in 0.1 ml PiCM was added to all tubes just prior to stimulation. Ten microliters of superoxide dismutase solution (Miles Biochemicals, Elkhart, Indiana), 1 mg/ml, was added to one tube in each triplicate to serve as control for nonspecific reduction of cytochrome C. The stimulus solution or PiCM (0.1 ml) was added at zero time to obtain a final volume of 1.0 ml in the stimulated and unstimulated reaction mixtures. After an additional 15 min at 37°C, the cell suspensions were centrifuged at 4°C. Reduced cytochrome C in the cold cell-free supernates was measured at 550 nm on a spectrophotometer (Model DU-7, Beckman Instruments, Novi, Michigan) and quantitated using the extinction coefficient 21.1 mm$^{-1}$.cm$^{-1}$ (reduced-oxidized). O$_2^-$ generation is expressed as nmol cytochrome C reduced per 1 × 10$^6$ PMNL per 15 min.

Lysozyme Release

Washed PMNL (3 × 10$^6$) in 0.9 ml PiCM were preincubated with serum (final concentration, 10%) for 15 min at 37°C followed by addition of 0.1 ml stimulus solution or buffer. After an additional 15
min, the cell suspensions were centrifuged and aliquots of the medium were assayed for lysozyme [6] and lactic dehydrogenase (LDH) activities. Cell viability was assessed by measuring leakage of the cytosolic enzyme LDH. Results are expressed as the percentage of total cellular enzyme released into the medium by Triton X-100 (0.2% v/v).

Phagocytosis

The rate of phagocytosis by PMNL of opsonized, lipopolysaccharide-coated paraffin oil droplets containing oil-red-0 was measured according to the method described by Stossel [7]. Results are expressed as milligrams of oil-red-0 ingested per min per 1 × 10⁷ PMNL.

Statistics

If not mentioned otherwise, mean values and standard deviations are shown. Data were analyzed by Student's t-test (two-tailed) where statistical significance was defined by a p value of less than 0.05.

RESULTS

Superoxide Anion Generation

Psoriatic PMNL + 10% autologous serum generated significantly less O₂⁻ compared to normal PMNL + 10% autologous serum (p < .001) when either PMA or Z was used as stimulus (Fig 1). Psoriatic or normal cells incubated with heterologous serum produced comparable intermediate levels of O₂⁻. Normal PMNL + heterologous psoriatic serum produced significantly less O₂⁻ than normal PMNL + autologous serum (p < .001), and O₂⁻ production was significantly increased for psoriatic PMNL + heterologous normal serum compared to psoriatic PMNL + autologous serum (p < .001). While variability is seen in the results of O₂⁻ generation among individuals (Fig 2), it should be noted that in every experiment (n = 17) the same trend was found: normal PMNL + normal serum > normal PMNL + psoriatic serum ≈ psoriatic PMNL + normal serum > psoriatic PMNL + psoriatic serum. There were no differences in baseline O₂⁻ generation in any of the four mixtures (Fig 1). Decreased O₂⁻ generation by psoriatic PMNL incubated with autologous serum compared to normal serum showed no correlation with the estimated extent of disease (Table I).

To evaluate whether the results are due to effects of the disease on serum or cells or both, a group of normal and psoriatic subjects' neutrophils were studied with autologous serum ± stimulus (Fig 3). There were no significant differences in O₂⁻ generation between normal and psoriatic PMNL without serum. When autologous serum is added, both types of cells demonstrate enhanced O₂⁻ generation; however, that of normal cells + serum is significantly increased compared to psoriatic PMNL + serum when either PMA or Z serves as stimulus.

To determine whether the effect of serum on O₂⁻ generation is time-dependent, we incubated normal and psoriatic PMNL with heterologous serum for variable time intervals from 0–60 min. In 8 experiments, no consistent effect of prolonged preincubation time was found (data not shown).

Lysozyme Release

Results paralleled those for O₂⁻ generation (Fig 4). Normal PMNL + autologous serum released significantly more lysozyme than psoriatic PMNL + autologous serum with either stimulus, p < .001. Differences between cells + autologous serum and cells + heterologous serum did not attain statistical significance. However, the same trend was obtained in each experiment (n = 7): normal PMNL + autologous serum > normal PMNL + psoriatic serum > psoriatic PMNL + normal serum > psoriatic PMNL + autologous serum. LDH release was less than 5% of total cellular enzyme in each experiment.
Phagocytosis

The rate of ingestion of oil-red-0 by normal PMNL (n = 14) was not different when compared to psoriatic PMNL (n = 8), 0.94 vs 0.98 mg/min/10^7 PMNL (Fig 5).

DISCUSSION

Previous investigations of PMNL function in psoriatic patients and the effects of their serum on function have had variable results. These differences appear to reflect study technique, patient population, and whether or not PMNL were preincubated with serum. Thus, PMNL of psoriatic patients have been reported to have increased [8] or normal [2,9] chemotaxis to endotoxin-activated serum, and these results did not correlate with activity of the disease.

Evaluation of the oxygen metabolism of PMNL from psoriatics has also produced divergent results. Using chemiluminescence as a measure of the generation of the reactive oxygen species, hydrogen peroxide, superoxide anion, hydroxyl radical, and singlet oxygen, Schopf et al [10] found enhanced generation of these products by both PMNL and monocytes of psoriatic patients. On the other hand, Camisa et al [11] found no difference in superoxide generation between psoriatics and normal controls as measured by superoxide dismutase inhibitable cytochrome C reduction. Resting nitroblue tetrazolium (NBT) reduction was normal for psoriatic PMNL, but 48-h cultures of peripheral blood mononuclear cells gave enhanced spontaneous NBT reduction [12]. When 10% normal plasma was added to these cultures NBT reduction was decreased by 50%, but addition of psoriatic plasma had no effect [12].

Sedgwick et al [13,14] also studied the effects of psoriatic serum on O_2^- generation by normal PMNL. They found increased and decreased O_2^- generation by normal cells stimulated by Z and PMA, respectively. The apparent difference in our results with zymosan can be explained by different methods of opsonizing zymosan.

In the present study, we attempted to reconcile the differences in reported results by studying several different PMNL functions in the presence and absence of serum using two different stimuli. We found that lysozyme release and superoxide generation were the same for psoriatic and normal PMNL in the absence of serum. An assay for PMNL phagocytosis that exploits activation of the alternative complement pathway in autologous serum gave comparable results for psoriasics and controls. However, in the presence of 10% autologous serum, lysozyme release and O_2^- generation were significantly decreased when psoriatic PMNL were compared to normals.

These data support the hypothesis that serum factor(s) exists in patients with psoriasis that may affect neutrophil functions. These factors may also play a role in the evolution and maintenance of the clinical manifestations of psoriasis. Further support for this concept derives from the recent demonstration that peritoneal dialysis can alter the course of refractory psoriasis [15]. If these hypothetical factors are not present in all psoriatic patients, the differences among the various studies of PMNL function may be explained on this basis.

Recent interest has focused on the role of arachidonic acid metabolites of the 5- and 12-lipoxygenase pathways, monohydroxyicosatetraenoic acids (HETEs), in the pathogenesis of psoriasis [16]. These highly chemotactic lipids have been found in scales [17], and soluble 5-lipoxygenase activity has been localized to the psoriatic epidermis [18]. Benoxaprofen, a potent inhibitor of 5-lipoxygenase, improved 75% of patients in a double-blind trial [19]. The effects of purified HETEs on PMNL function have not yet been completely defined, but they did slightly inhibit baseline superoxide generation [20] and phagocytosis-associated chemiluminescence [21] at chemotactic concentrations. Whether elevated levels of HETEs in psoriatic serum could explain our observations is currently under investigation.

REFERENCES


Fig 4. Percent release lysozyme by normal and psoriatic PMNL incubated with 10% autologous or heterologous serum after stimulation with PMA or opsonized zymosan.

Fig 5. Rates of ingestion of lipopolysaccharide-coated paraffin oil droplets pretreated with autologous serum by PMNL of normal controls and patients with psoriasis.