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Polymorphonuclear Leukocytes: Possible Mechanism of Accumulation in Psoriasis

Abstract. *Extracts of involved and uninvolved skin from nine patients with untreated psoriasis were studied for chemotactic activity. Psoriatic plaque contains increased amounts of a complement-dependent chemotactic factor that is inhibited by diisopropyl fluorophosphate. This factor may be human skin serine proteinase.*

Psoriasis is a common skin disease that is characterized clinically by sharply circumscribed, scaly red plaques. The epithelium of psoriatic lesions has an increased mitotic rate (*1*). One of the very early characteristic histological features is the presence of small microscopic foci of polymorphonuclear leukocytes (PMN's) in the stratum corneum (*2*). Tagami and Ofuji have shown that psoriatic scale contains leukotactic substances that may be products of complement activation (*3*). Proteinases extracted from tissue and cells are able to generate chemotactic peptides from complement (*4–6*). Several laboratories have demonstrated that psoriatic scale has an increased content of proteolytic enzymes (*7*). The experiments described here demonstrate that psoriatic plaque epidermis contains increased amounts of a serine proteinase which activates complement and induces PMN accumulation.

Elliptical biopsies (2.5 by 1 cm) of the rim of clinically active, nonpustular psoriatic plaques and normal appearing surrounding tissue were obtained under sterile conditions from the gluteal or thigh area of nine untreated psoriatic patients (*8*). Tissues were also obtained from five normal control subjects and one patient with pityriasis rubra pilaris. Sections of all specimens were removed for histological examination. The tissues were immediately trimmed of subcutaneous fat and placed in 2*M* KBr at 37°C for 30 minutes; the epidermis was easily removed from the underlying dermis by gentle traction (*9*). The epidermis preparations were washed five times in phosphate buffered saline (2 hours, 4°C) and then placed in 50 m*M* phosphate buffer, *p*H 7.5, containing 1*M* KCl and frozen and thawed five times. The tissue was extracted for 16 hours with gentle shaking at 4°C. The soluble extract was clarified by centrifugation (50,000*g*, 20 minutes, 4°C) and the extracted tissue pellet was frozen and assayed for DNA content (*10*). We have used the KBr epidermal preparation technique in previous work (*9*) and have documented that proteinase recovery is quantitative.

The clear supernatant solution was assayed for protein (*11*), the lysosomal proteinase cathepsin D (*9*), and neutral proteinase (*9*). The extract was then dialyzed against sterile saline and adjusted to a final protein concentration of 0.5 mg/ml. Chemotactic activity of the extracts was measured by assaying the accumulation of PMN's in the peritoneal cavity of mice according to the method of Snyderman *et al.* (*12*). Samples (2 ml) were injected into the peritoneal cavities of groups of normal and male mice deficient in the fifth component of complement, C5 (Jackson Laboratory). After 12 hours the animals were killed by inhalation of CO_2, and the peritoneal cavity was washed vigorously with 9 ml of Dulbecco's modified Eagle's medium with 10 percent fetal calf serum (Gibco) containing heparin (10 unit/ml). The peritoneal wash was counted for total number of white cells and for the percentage of PMN's by standard techniques. Results are expressed as absolute numbers of PMN's. At least two mice were injected with extracts of normal and psoriatic tissue from each of the nine patients. We had sufficient tissue in five patients to take portions of extract and incubate our preparations with 1 m*M* diisopropyl fluorophosphate (DFP) for 2 hours and then dialyzed them against three changes of physiological saline (12 hours, 4°C) before injection into the mice. Psoriatic extract was injected into three normal and three C5-deficient mice (*6*, *13*) from each of three patients. All chemotaxis experiments also included the injection of three mice with physiological saline, and the injection of three mice with proteose peptone (9 percent) (Difco), a complement-independent chemotactic agent. The chemotactic assay was demonstrated to be dose-dependent by injecting various dosages of purified proteinase into groups of three mice on three separate occasions (*14*).

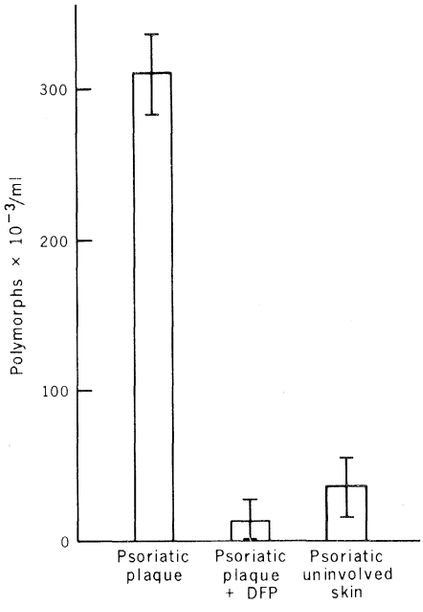

Fig. 1. Polymorph accumulation induced by injection of extracts of involved and uninvolved psoriatic skin (1 mg of protein per mouse) from nine patients. Results are expressed as the mean ± 1 standard error ($P < .01$); *DFP*, diisopropyl fluorophosphate.

Extracts of psoriatic plaque induced the accumulation of PMN's in the peritoneal cavity of normal mice (Fig. 1). This activity could be almost completely inhibited by prior incubation of the extracts with the serine proteinase inhibitor, DFP. Extracts of involved psoriatic skin induced significantly more accumulation of PMN's than extracts of uninvolved tissue ($P < .01$). Extracts of uninvolved psoriatic epidermis were slightly more effective in inducing PMN accumulation than extracts of control epidermis or epidermis from the patient with pityriasis rubra pilaris. Injection of equal amounts of psoriatic extract into normal and C5-deficient mice demonstrated that the enzyme evoked the ex-

pected dramatic response in normal mice but was only one-quarter as effective in evoking PMN infiltration in the C5-deficient mice ($P < .05$) (Fig. 2). Proteose peptone, a complement-independent chemotactic agent, evoked similar numbers of polymorphonuclear leukocytes in the peritoneum of normal and C5-deficient mice. These data demonstrate that psoriatic tissue evokes its chemotactic response primarily through the complement cascade and requires the production of C5 cleavage products for maximum activity.

Approximately 10 percent of the wet weight of both normal and psoriatic epidermis was extractable as soluble protein. The specific activity of neutral proteinase activity, expressed as units of enzyme activity per milligram of protein in the extract, was 1.3- to 2.5-fold higher in involved psoriatic plaque than in noninvolved epidermis. No correlation between total amounts of neutral proteinase activity and chemotactic activity could be established in our limited number of patients. By contrast, the specific activity of the lysosomal proteinase, cathepsin D, was similar for both involved and uninvolved skin. Normalization of data for the DNA content of our extracted epidermis did not change our results significantly.

Histological examination of the psoriatic plaques revealed epidermal hyperplasia, very minimal round cell infiltration of the dermis, and sparse accumulations of several PMN's in the parakeratotic stratum corneum. No specimen demonstrated substantial numbers of PMN's infiltrating the epidermis.

Our data suggests that psoriatic plaque contains a chemotactic factor that is inhibited by DFP and operates by proteolytic activation of complement. By contrast, noninvolved psoriatic epidermis and control epidermis contain significantly less of this chemotactic factor. Pityriasis rubra pilaris (PRP) is strikingly similar to psoriasis in its clinical picture and increased mitotic rate (*15*). By contrast, it does not have PMN accumulation in the epidermis. It is notable, therefore, that the extract from the patient with PRP did not attract more PMN's than normal control epidermis. It is reasonable to consider the possibility that this factor is similar to the chemotactic neutral proteinase which we have purified from human skin (*6, 16*), separated human epidermis (*9*), human lymphocytes (*17*), and mouse epidermal cells in culture (*14*). This enzyme has a molecular weight of 28,000, is inhibited by DFP, α_2-macroglobulin, and soybean trypsin inhibitor, and requires the presence of C5 to induce PMN accumulation (*6, 16*). It is very unlikely that chemotactic activity in psoriatic epidermis is a function of PMN accumulation since trivial numbers of PMN's are seen histologically, much of the stratum corneum, where the PMN accumulation is found, is lost during the KBr separation procedure, and there is no increase in the specific activity of acid proteinase (an enzyme found in PMN's) in psoriatic epidermis. The specific activity of neutral proteinase was higher in involved tissue than in uninvolved tissue in all nine of our patients. Exact correlation of chemotactic activity with neutral proteinase levels must await refinement of the immunoassay procedure because our proteolytic assay, which is based on the degradation of [^{3}H]casein to a trichloroacetic acid–soluble peptide, measures several proteinases in addition to chemotactic proteinase in crude skin homogenates. Exact determination of the proteinase responsible for PMN accumulation in psoriasis requires isolation and characterization of proteinase from psoriatic tissue. The specific activity of the lysosomal proteinase, cathepsin D, was similar in involved and uninvolved psoriatic skin. This observation further supports the hypothesis that chemotactic proteinase is specifically increased in psoriatic epithelium.

Our observation may explain the presence of complement cleavage products in the stratum corneum of psoriatic plaques (*3, 18*) because we have shown that psoriatic plaque contains increased amounts of a serine proteinase which activates complement and induces PMN accumulation. This proteolytic activity, along with arachadonic acid metabolites (*19*), could explain the accumulation of PMN's in the epidermis in psoriatic plaques. We do not know whether chemotactic proteinase is primary to the psoriatic process but our studies suggest that increases in chemotactic proteolytic activity in epithelial cells may be a possible mechanism for PMN accumulation. Indeed, increases in chemotactic proteolytic activity in a wide variety of tissues could explain PMN accumulation in a number of pathological conditions.

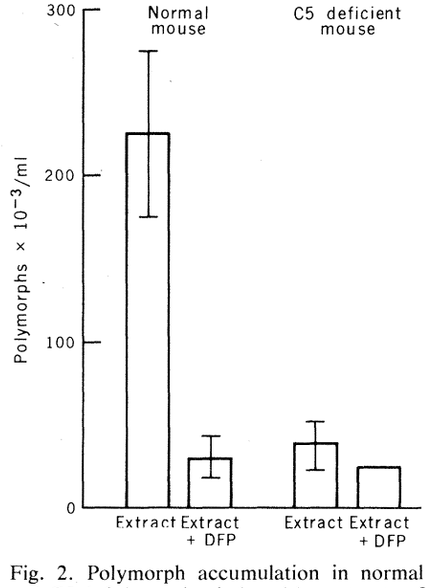

Fig. 2. Polymorph accumulation in normal and C5-deficient mice induced by extracts of psoriatic plaques (1 mg of protein per mouse) from three different patients. Results are expressed as the mean ± 1 standard error ($P < .05$); *DFP*, diisopropyl fluorophosphate.

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