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Neutrophil Leukocyte Migration in Psoriasis Vulgaris

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Neutrophil migration in patients with psoriasis vulgaris was assessed using both *in vitro* and *in vivo* techniques. *In vitro* chemotaxis towards endotoxin-activated serum and casein was found to be normal; however, random filter migration was increased in patients with extensive lesions. No significant difference was observed between the effects of psoriatic and normal serum on control leukocyte *in vitro* migration. *In vivo* skin window chamber random migration and chemotaxis towards 50% autologous serum were also found to be normal in uninvolved skin, regardless of disease severity.

We have not been able to confirm previous reports of abnormal leukocyte chemotaxis in psoriasis, and submit that neutrophil migration into psoriatic plaques *in vivo* is the result of a local accumulation of chemotactic factors rather than of enhanced leukocyte chemotactic activity.

Transepidermal leukocyte migration and the resultant accumulation of neutrophil polymorphs in Munro's microabscesses in the stratum corneum are characteristic histological features of psoriasis [1]. Polymorphonuclear leukocytes contain hydrolases that induce cell division *in vitro*, and their presence in psoriatic lesions may further exacerbate the primary abnormality of accelerated epidermal cell division [2]. A knowledge of the factors responsible for this neutrophil migration is likely to be of considerable importance in helping our understanding of the pathogenesis of psoriasis. Psoriatic plaques and scale extracts have been shown to contain leukotactic substances including immune complexes, complement breakdown products, arachidonic acid oxidation metabolites and bacterial chemotactic factors [3–10]. However, reports on intrinsic neutrophil leukocyte chemotactic activity in psoriasis have been conflicting. We have therefore studied neutrophil migration in psoriatic subjects both *in vitro* by a modified Boyden chamber method, and in uninvolved skin *in vivo* using a quantitative skin window chamber technique.

MATERIALS AND METHODS

Subjects

Fifty patients with psoriasis vulgaris who were free of other disease and not receiving any systemic medication and 50 healthy subjects obtained from hospital staff and students were studied. Informed consent was obtained from each individual. Twenty-seven of the patients were male and 23 were female, while 26 of the control subjects were male and 24 were female. The age of the patients ranged between 18 and 73 yr (mean 37), while that of the control subjects ranged between 19 and 69 yr (mean 28). Twenty-five of the psoriatics had extensive plaque psoriasis with 25% or more of the body surface area involved and were receiving in-patient dithranol therapy; the remainder were outpatients with only minor skin involvement (10% or less of the body surface area involved) being treated with a variety of topical medications including dithranol, weak topical corticosteroids and tars.

In Vitro Chemotaxis

A modification of Wilkinson's method was used [11–13]. Washed neutrophil suspension containing 2×10^6 cells/ml prepared from dextran-sedimented whole blood was introduced into polystyrene tubes, to the lower ends of which had been glued 3 μ pore size filters (Millipore U.K. Ltd.). The tubes were then placed into wider bore tubes containing chemotactic solution. Migration assays were conducted simultaneously in pairs; each pair contained neutrophil suspensions from one psoriatic and one healthy control subject. The effect of sera from normal persons and from patients with psoriasis on control cell migration was investigated in a second series of experiments. Washed normal neutrophil suspension was preincubated for 30 min at 37°C in the presence of either 10% psoriatic or 10% autologous freshly collected untreated serum before introduction into the chemotaxis chambers. Chemotactic solutions consisted of [1] casein 1 mg/ml in Hank's balanced salt solution and [2] endotoxin-activated serum prepared by mixing 0.2 ml endotoxin (lipopolysaccharide type II, Sigma) with 0.4 ml fresh serum and 4.4 ml Hank's balanced salt solution. Hank's balanced salt solution alone was used as a control. After a 45 min incubation period at 37°C, filters were removed, fixed and stained, and the distance migrated by the leading front of cells through the depth of the filter was measured from the surface of the filter using a calibrated microscope. Each test was performed in duplicate, and 4 readings were taken from each filter, so that results were expressed as a mean of 8 readings.

In Vivo Chemotaxis

A quantitative skin window chamber technique [12, 13] was employed to assess *in vivo* chemotaxis in 48 psoriatics and 37 normal volunteers. Four small superficial abrasions were made on uninvolved skin of the flexor surface of the forearm with a dental burr mounted in a hand-held drill. Following haemostasis, plastic chambers of capacity 0.6 ml were cemented over the cleaned abraded wounds with Powabond adhesive (Staident Products Ltd.). Chemotactic solution was introduced into the chambers through a hole in the top which was then stoppered. Each test was performed in duplicate. One pair of chambers contained Hank's balanced salt solution as a control, and the other pair contained Hank's balanced salt solution with 50% freshly collected untreated autologous serum, 90% of the chemotactic activity of which is due to the complement fraction C_5 [14]. After 24 hr, fluid in the chambers was aspirated and the cell content was measured using a hemocytometer. The abrasion areas were determined by a photographic method. The cell count per 10 sq mm of abrasion per 24 hr was then calculated, results being expressed as a mean of those from each pair of duplicate chambers.

Statistical Methods

The results of the experiments on *in vitro* and *in vivo* chemotaxis were evaluated by Student's *t*-test (2-tailed). The effects of preincubation in either 10% psoriatic or control serum on control cell *in vitro* migration were evaluated by the paired *t*-test.

RESULTS

In Vitro Migration

No significant difference was observed in either random migration towards Hank's buffer alone or in chemotaxis towards endotoxin-activated serum or casein between the 50 psoriatics and the equal number of controls (Fig 1). Comparison of the 25 cases with extensive plaque psoriasis with their corresponding controls again revealed no significant difference in chemotaxis towards endotoxin-activated serum and casein (Fig 2). How-

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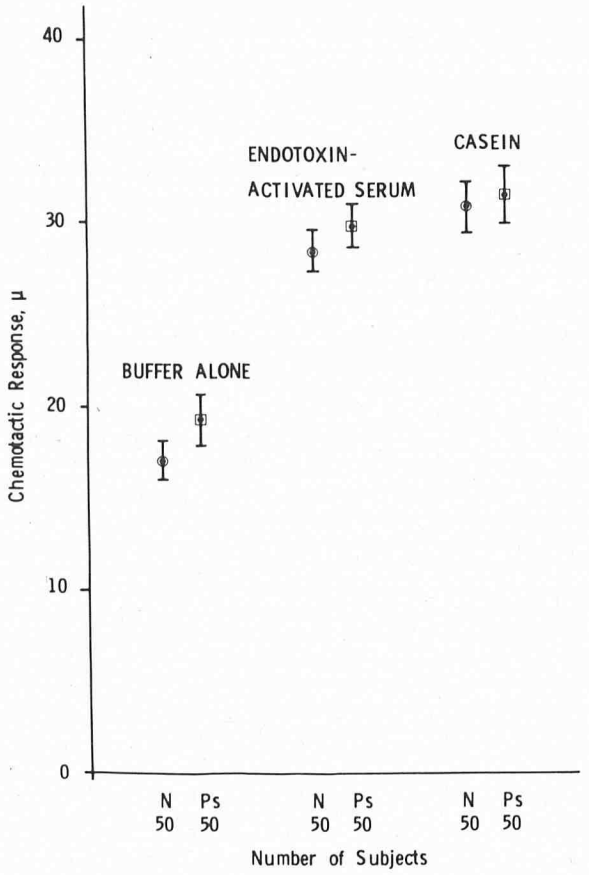

FIG 1. *In vitro* neutrophil random migration (buffer alone) and chemotaxis towards endotoxin-activated serum and casein. All values mean ± SEM. No significant difference between the 50 pairs of control (*N*) and psoriatic (*Ps*) subjects was observed.

ever, an increase in random migration towards Hank's buffer alone by neutrophil leukocytes from patients with extensive psoriasis was statistically significant ($p < 0.02$).

Effect of Psoriatic Serum on Control Cell in Vitro Migration

Preincubation in both 10% psoriatic and 10% autologous serum produced an increase in random migration and chemotaxis towards endotoxin-activated serum and casein by control neutrophil leukocytes *in vitro* (Fig 3). No significant difference was observed between the effects of autologous serum and of psoriatic serum, even when using serum obtained from the 25 cases with extensive plaque psoriasis. Sera from patients with extensive psoriasis did not increase control cell random migration to a greater extent than did normal sera.

In Vivo Migration

More than 90% of the migrating cells were neutrophil leukocytes in every case. No significant difference was demonstrated in either random migration towards Hank's buffer alone in the chamber, or in chemotaxis towards 50% autologous serum in the chamber, between the 48 psoriatic patients and the 37 normal subjects (Fig 4). Random migration was not increased *in vivo* in the 25 cases with extensive plaque psoriasis. An apparent decrease in chemotaxis towards 50% autologous serum in the patients with extensive psoriasis was not statistically significant ($p > 0.05$).

DISCUSSION

Reports on intrinsic neutrophil chemotactic activity in psoriasis have been conflicting. Glinski et al [15] reported decreased *in vitro* random migration using a capillary tube method, but most workers using filter migration techniques have found it to be normal [5,16,17]. We also found no overall significant difference in random filter migration between 50 psoriatics and 50 normal subjects (Fig 1). However, a subgroup of 25 patients with extensive psoriasis manifested a statistically significant increase in filter random migration when compared with their corresponding controls (Fig 2). This increase in random migration would not appear to be the result of an intrinsic abnormality of psoriatic leukocytes as it was not also observed in the subgroup of patients with less extensive psoriasis. Although the increase in random leukocyte migration in patients with severe psoriasis may be secondary to the disease, it would not seem immediately attributable to a serum-induced effect as sera from patients with extensive psoriasis did not increase control cell *in vitro* random migration to a greater extent than did normal sera (Fig 3).

Although several groups have reported normal *in vitro* neutrophil chemotaxis in psoriasis [5,17], Wahba et al [16,18] in a large series of 52 patients with psoriasis found significantly enhanced neutrophil chemotaxis towards endotoxin-activated serum in all but 7 cases, regardless of extent of skin involvement. Plasma from 4 patients with extensive lesions markedly depressed the chemotactic activity of control neutrophil leukocytes [18]. They concluded that there is an intrinsic abnormality of neutrophil leukocyte function in psoriasis, possibly related to a decreased intracellular cyclic AMP: cyclic GMP ratio [19], and that plasma factors in psoriatic subjects influence chemotactic responsiveness and are related to disease extent. We were unable to demonstrate any increase in neutrophil chemotaxis towards endotoxin-activated serum or casein in 50 patients with psoriasis, regardless of disease extent (Fig 1 and 2). Similarly, no significant difference between the effects of psoriatic serum,

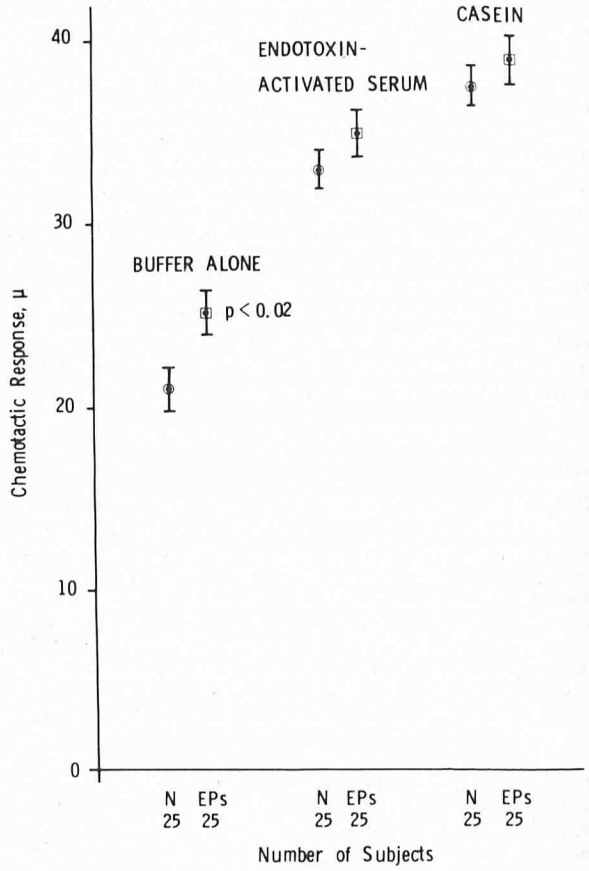

FIG 2. *In vitro* neutrophil random migration and chemotaxis in a subgroup of 25 psoriatics with extensive disease (*EPs*) and their corresponding controls (*N*). All values mean ± SEM. An increase in random migration but not in directed chemotaxis in the psoriatic group reached statistical significance (Student's *t*-test, 2-tailed).

even when taken from 25 cases with extensive lesions, and normal serum on control neutrophil leukocyte *in vitro* chemotaxis was observed (Fig 3). The discrepancy between our results and those reported by Wahba et al [16,18] may be due in part to technical differences in the procedures used to assess chemotaxis. These authors employed the "lower surface count" method, whereas the "leading front" method, which has been shown to consistently produce more accurate and more reproducible results [20], was used in our experiments.

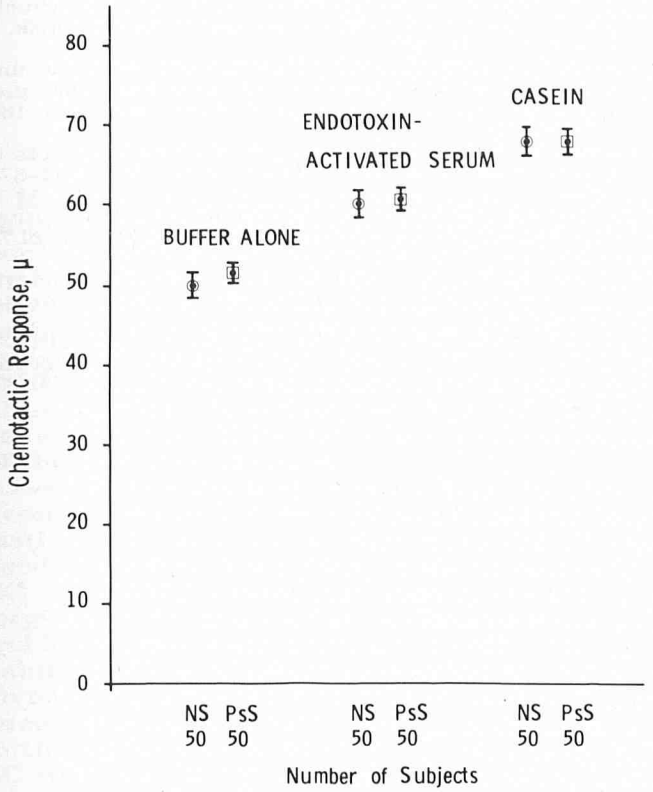

FIG 3. Effect of pre-incubation in either 10% psoriatic serum (*PsS*) or in 10% autologous serum (*NS*) on control neutrophil *in vitro* migration. All values mean ± SEM. No significant effect of psoriatic serum on control neutrophil random migration or chemotaxis was demonstrated.

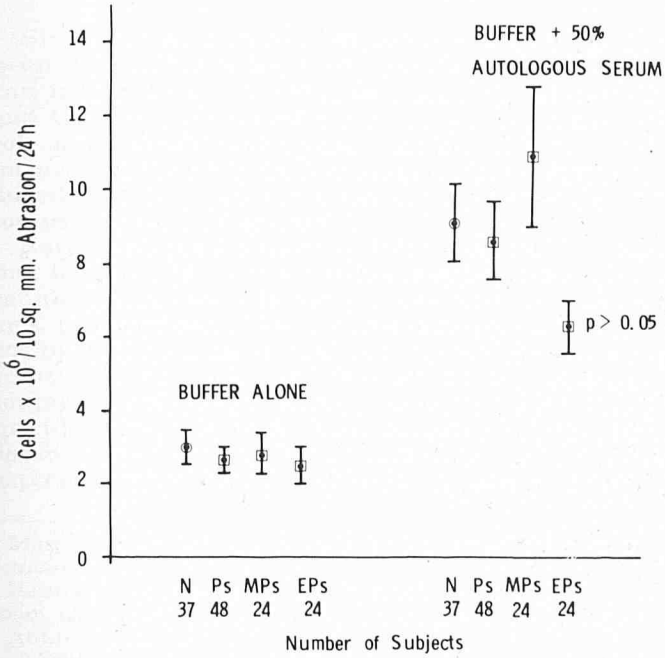

FIG 4. *In vivo* skin window chamber migration in uninvolved skin; response to Hanks buffer alone (random migration) and to 50% autologous serum. All values mean ± SEM. *N* = controls, *Ps* = all psoriatics, *MPs* = subgroup with minor disease, *EPs*=subgroup with extensive disease. No significant difference in migration between the control group and any psoriatic group or subgroup was observed (Student's *t*-test, 2-tailed).

There have been few previous reports on *in vivo* leukocyte migration in psoriasis. Studies involving the use of the Rebuck technique have found normal migration in uninvolved skin but decreased migration in involved skin [21], and normal or decreased migration in involved skin [22]. However, accurate quantification of cell migration is difficult with this method. Von Muffel, Kluge, and Ruffert [23], using a quantitative skin window chamber technique similar to that used in our experiments, reported decreased migration in uninvolved skin in 23 patients. We found that *in vivo* neutrophil random migration and chemotaxis towards 50% autologous serum were normal in uninvolved skin in 48 patients with psoriasis, regardless of extent of disease (Fig 4). *In vivo* migration in involved skin was not investigated due to problems with adherence of the chambers to psoriatic plaques.

Reports of increased neutrophil nitroblue tetrazolium reduction [24], increased granulocyte superoxide generation and adherence to nylon fiber columns [25,26] and the findings of Wahba et al [16,18] of enhanced neutrophil chemotactic activity and phagocytosis have led to a theory that circulating leukocytes in psoriasis are "activated" and therefore readily attracted by chemotactic stimuli [2]. We have not been able to confirm previous reports of an intrinsic abnormality of neutrophil chemotaxis in psoriasis either *in vitro* or *in vivo*, and submit that neutrophil migration into psoriatic plaques *in vivo* is the result of a local accumulation of chemotactic factors. A suggestion of possible therapeutic benefit in psoriasis from using drugs which influence leukocyte migration [2] may be somewhat premature.

Addendum

Since this article was submitted for publication Silny and colleagues [27], using a modified Boyden chamber technique and the lower surface count method, have reported a significant increase in polymorphonuclear leukocyte chemotaxis in patients with psoriasis vulgaris before and after oral photochemotherapy (PUVA).

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