Dapsone and Human Polymorphonuclear Leucocyte Chemotaxis in Dermatitis Herpetiformis

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Polymorphonuclear leucocyte chemotaxis was investigated in 6 patients with active dermatitis herpetiformis in the untreated state and when under control with Dapsone. Control studies were undertaken in 7 healthy volunteers. No significant difference in chemotaxis was demonstrated between the active, treated and control groups. Furthermore, when Dapsone in physiological concentrations was added separately in all three groups there was no additional effect on chemotaxis. These results clearly show that polymorphonuclear leucocytes from active untreated dermatitis herpetiformis have normal chemotactic activity compared with those from the same patients in the treated state and controls. Additional Dapsone did not alter these findings. Key words: Dermatitis herpetiformis; Dapsone; Polymorphonuclear cells; Chemotaxis. (Received February 15, 1984.)

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Dermatitis herpetiformis (DH) has a characteristic histological lesion which shows accumulation of polymorphonuclear leucocytes (PMN) in the tips of the dermal papillae as a prominent feature. This accumulation of PMN is in response to chemotactic factors generated by an as yet ill-defined immunological event. Dapsone (DDS) causes rapid resolution of clinical and histological lesions with disappearance of PMN from the dermal papillae. DDS modifies PMN behaviour in other conditions which include sub-corneal pustular dermatosis, erythema elevatum diutinum, and occasionally pyoderma gangrenosum, leukocytoclastic vasculitis, and pustular psoriasis.

This study, with treated cases and controls, was conducted to study PMN chemotaxis and the effect of DDS on chemotaxis.

MATERIALS AND METHODS

Patients. Six patients with established DH were investigated (5 males, 1 female, mean age 53 years) and 7 healthy volunteers (4 males, 3 females, mean age 42 years). The DH patients exhibited characteristic histopathological criteria of the condition, and direct immunofluorescence when undertaken showed granular IgA deposits in the dermal papillae.

The patients were treated with DDS (daily dose 30-100 mg) sufficient to control expression of the disease. Informed consent was given for the cessation of DDS treatment for one week (all the patients developed active blistering after this period). One patient was taking a gluten-free diet. No patients had illness or drug therapy known to affect leucocyte function.

Chemotaxis assay

PMN were obtained by sedimentation of heparinised venous blood with mono-poly resolving medium. The plasma and mononuclear leucocyte layer was removed and discarded. The PMN layer was collected, washed, and centrifuged. The resulting pellet was resuspended in 5 ml of medium 199 and a cell count performed. After a final wash an appropriate volume of medium 199 was added to give a final cell concentration of $1 \times 10^7$ PMN/ml. Cell recovery was 30-50% and viability >90% as assessed by the Trypan Blue exclusion technique.

N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP) was used as the chemo-attractant in a concentration of $10^{-6}$ M in Medium 199. Suspensions of PMN containing $5 \times 10^6$ PMN/ml were prepared with varying concentrations of DDS from a stock solution of 100 µg/ml in Medium 199 to give final concentrations of 4 µg/ml, 20 µg/ml, and 40 µg/ml. A control suspension contained no DDS.
Fig. 1. Tissue culture dish with agarose medium. Four sets of wells 3 mm in diameter and 3 mm apart.

Fig. 2. Diagrammatic representation of method used to determine the number of cells migrating under agarose towards FMLP.

Agarose plates containing 0.5% Agarose, 10% Foetal Bovine Serum, 20 mM Hepes Buffer, and 2 mM L-glutamine in Medium 199, were freshly prepared. 3 mm wells were cut in four pairs 3 mm apart in each plate (Fig. 1). The Agarose plugs were removed by a vacuum technique. 3 µl of 10^-6 M FMLP was added to the outer wells and 3 µl of 5×10^6 PMNL/ml suspension with or without DDS was added to the inner wells. The plates were covered and incubated at 37°C in a humified atmosphere for 90 min. The chemokinetic effect was accounted for by observing random migration from wells containing PMN in isolation from chemotactic factors. Cell migration was counted using an inverted microscope at ×50 magnification with a boxed eyepiece grid aligned to the circumference of the well. The two central columns were counted as they were found to represent full field migration (Fig. 2). Dishes were set up in duplicate. The mean of those readings was made for each observation. Results were expressed as directional migration scores ± standard error of the mean.

RESULTS

The mean chemotactic migration scores are given in Table I. Comparisons between like groups were made according to the paired t-test and comparisons with the control group were made according to the two sample t-test. The null hypothesis was not disproved in any of these comparisons. The narrow band of comparability between the mean migration scores is shown (Fig. 3). These results indicate functional similarity between DH (treated and untreated) and control PMN and no apparent Dapsone effect at each concentration within the range of accepted therapeutic effect.
Esca et al. (1) studied the chemotactic response to activated serum of PMN from DH patients and matched controls. The DH patients were all on sulphone treatment and no difference was found between the two groups, thus suggesting that the DH PMN is not unduly responsive to chemotactic factors. However, the observation remains that PMN are the dominant cell of the high dermal infiltrate in active DH and that treatment with DDS and other sulphones results in their rapid disappearance. Standahl et al. (2) showed that PMN from normal individuals do not exhibit altered chemotactic activity in the presence of DDS and that the DDS effect lies rather in the depression of myelo-peroxidase mediated cytotoxicity. This observation does not explain the rapid action of DDS in DH on mobilisation of the PMN.

We thought it important to study the circulating PMN from untreated DH patients in respect of chemotactic function and susceptibility to DDS effect in a full range of added concentrations. We compared results with those of PMN from treated DH patients and from normal individuals so as to maximise the experiment. The 1-week period off active treatment with development of clinical disease activity was thought to be sufficient to eliminate DDS effect. The mean plasma half-life of 24 hours (3) and clinical observations suggest this drug-free period to be sufficient.

The uniformly similar comparisons in our series suggest that the PMN of patients suffering from active DH have normal chemotactic behaviour but do not imply that DDS

**Table I. Table of results**

<table>
<thead>
<tr>
<th>DDS ...</th>
<th>0 µg/ml</th>
<th>4 µg/ml</th>
<th>20 µg/ml</th>
<th>40 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH untreated</td>
<td>89.3±18.2</td>
<td>81.0±22.9</td>
<td>91.8±24.7</td>
<td>84.0±22.1</td>
</tr>
<tr>
<td>DH treated</td>
<td>73.1±16.6</td>
<td>68.5±17.4</td>
<td>92.8±13.5</td>
<td>80.2±18.6</td>
</tr>
<tr>
<td>Control</td>
<td>83.7±16.0</td>
<td>69.3±15.9</td>
<td>68.3±9.1</td>
<td>77.9±16.6</td>
</tr>
</tbody>
</table>

**Fig. 3. Effect of Dapsone on P.M.N.L. Migration to F.M.L.P.**
has no effect on PMN chemotaxis in vivo. Further investigation will explore the effect of DDS metabolites and will endeavour to identify specific DDS-influenced chemotactic factors.

ACKNOWLEDGEMENT

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REFERENCES


Effect of Grenz Rays on Langerhans' Cells in Human Epidermis

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In order to investigate the effect of grenz rays on Langerhans' cells in human epidermis, five healthy volunteers were treated with a single dose of 4 gray of grenz rays on a limited area of buttock skin. Biopsies were obtained before irradiation and from the irradiated site 30 min, 6 hours, 24 hours, 1 week and 3 weeks after X-ray therapy. There was a slight reduction after 30 min and a very pronounced reduction of OKT-6 positive cells regarded as Langerhans' cells, 1 and 3 weeks after irradiation.

Key words: Bucky rays; Langerhans' cell; Monoclonal antibody.

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It is only within the past 5 years that the important role of Langerhans' cells in the immune system of epidermis has been recognized, though these cells have been known for a long time. For summary see (1).

A large amount of work has been performed concerning the effects of ultraviolet light on Langerhans' cells. As regards X-rays on the other hand, little is known (2-5). In mice soft X-rays reduce Langerhans' cells in a dose-dependent manner (4, 5).

Long wave X-rays for the treatment of cutaneous diseases have now been used for more than 60 years after Bucky's introduction of grenz rays. The X-rays of long wavelength used in dermatology are the following: Soft X-rays (average wavelength 0.015 nm), superficial X-rays (average wavelength 0.05 nm) and grenz rays (average wavelength 0.2 nm). Synonyms for grenz rays are Bucky rays and ultrasoft rays and those are the waves closest to the ultraviolet light in the spectrum of electromagnetic radiation.

The aim of the present study was to investigate the effect of a single dose of grenz rays on the number of OKT-6 positive cells in normal human skin. The dose of grenz rays selected was 4 gray which is a dose commonly used in the treatment of benign skin disorders.