Inhibition of PMN Leukocytes Chemotaxis by Thalidomide

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Summary. The effects of thalidomide on chemotaxis of normal human peripheral blood PMN leukocytes have been studied in vitro. The chemotaxis factor was generated by interacting normal human serum with bovine gamma globulin-antibovine-gamma globulin immune complexes. At concentrations of 1, 10, and 100 μg/ml, thalidomide failed to inhibit the chemotactic factor. At the same concentrations, erythromycin caused a marked inhibition of chemotaxis. Pre-incubation of PMNs with thalidomide or erythromycin caused a marked, dose-independent inhibition of chemotaxis. Random mobility did not appear to be affected.

Inhibition of PMN chemotactic ability by thalidomide may account for its ability to improve inflammatory dermatoses, such as aphthosis.

Key words: Aphthosis — Chemotaxis — Erythromycin — Leukocytes — Thalidomide


Die Inhibition der chemotaktischen Fähigkeit polymorphonukleärer Leukocyten, verursacht durch Thalidomid, könnte für die Potenz dieses Medikamentes sprechen, entzündliche Dermatosen, wie die Aphtose, günstig zu beeinflussen.

Abbreviation: MEM: Minimal Essential Media

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Oral administration of thalidomide has been successfully used empirically to treat various cutaneous inflammatory diseases, such as discoid lupus erythematosus, pyoderma gangrenosum, erythema multiforme, and aphtosis (unpubl. pers. commun. and [5]). The basis for this anti-inflammatory action is not known. However, inhibition of leukocyte chemotaxis by a drug could account, partly, for its efficacy in the treatment of skin disorders associated with dermal accumulation of leukocytes. To study whether this mechanism could account for the beneficial effect of thalidomide in aphtosis, we have examined its effect on polymorphonuclear (PMN) leukocytes chemotaxis.

PMN chemotaxis with different concentrations of Thalidomide was studied in vitro and compared with that obtained with erythromycin, a drug which, in the same conditions, has previously been shown to inhibit chemotaxis [2].

**Material and Methods**

**Material**

Cell and sera were obtained from healthy human male volunteers, receiving no drug. Sera and cellular suspensions were prepared in a sterile fashion.

**Cell Preparations**

Thirty milliliters of venous blood was collected in a heparinized tube on the day of assay. The blood was diluted with an equal volume of 3% Dextran (Dextran, Clin., Macrodex. Clin-Comar-Byla), in physiological serum and allowed to sediment for 60 min at 37°C. The supernatant was centrifuged for 10 min at 250 × g. The erythrocytes were removed from the cellular deposit by lysis with ammonium sulfate (0.8%). After washing with MEM (Gibco, Grand Island, NY, USA) the granulocyte suspension was adjusted to 4 × 10^6 cells/ml in MEM. Cell viability was 99% by trypan blue exclusion.

**Chemotactic Factor**

Chemotactic factor was prepared from fresh normal human serum by the method of Leung-Tack et al. [4]. Briefly, serum was diluted 1/10 with MEM and the principal complement pathway activated by incubating it for 30 min at 37°C with guinea pig antibody-antigen immune complexes (IC) (300 μg of antibody; antibovine gammaglobulin/bovine gammaglobulin ABGG/BGG). Activated serum was then centrifuged for 10 min at 400 × g. The supernatant was incubated for 30 min at 56°C.

**Migration Chambers**

Migration chambers and chemotactic assays have been described previously [4]. Briefly, the lower compartment, a beaker, contained two cylindrical chambers that were made by cutting syringes, and represented the upper compartments. Millipore filters (millipore SSWP 02500, pore 3 μm) were glued to the bottom of these cylinders and inserted between the two compartments.

**Preparation of Drugs**

Thalidomide (thalidomid-Grünenthal, FRG) and erythromycin (Roussel, France) were tested. Stock solutions were prepared in MEM. Each drug was assayed in concentrations of 1 μg/ml, 10 μg/ml, and 100 μg/ml.

**Chemotaxis Assays**

Random mobility was studied with 2 ml of 10% serum only in the lower compartment. Chemotaxis assays were performed using activated serum (10% serum activated with ABGG/BGG IC) as the attractant medium, 2 ml in the lower compartment.