Since alteration of *maximally-stimulated* PMN movement would be the most sensitive method for detecting inhibitors or enhancing substances, we have attempted to maximize PMN migration responses to a defined stimulus, the synthetic chemotactic formyl-peptide F-met-leu-phe (FMLP). When PMNs isolated from human blood are placed in wells cut in agarose gels containing FMLP and any additives under study, PMNs migrate *radially* from the wells into the potential space between gel and plate. Migration is interrupted by glutaraldehyde fixation at 2, 4 and 18 hours to permit calculation of *peak rates* and *final distances* of translational migration for leading front PMNs.

Peptide-stimulation alone produced rapid initial rates (8-12 µm/min) but addition of albumin and γ-globulin in high concentration (2 x 10^{-6}M) or of serum in low concentration (4% v/v) markedly enhanced both peak rates and final distances of peptide-stimulated radial migration. Furthermore enhanced responses reduced variations between donors and permitted definition of normal ranges for peak migration rates and final distances.

The agarose radial migration assay is simple to perform and read, and yields reproducible individual responses (~5%). Quantitative studies characteristically result in steep dose-response curves when migration is inhibited by agents known to interfere with PMN motility, such as inhibitors of Na^+-K^+-ATPase, anaerobic glycolysis and microfilament function. These results all indicate that alteration of rates of *maximally-stimulated radial* migration of PMNs is the most sensitive screening technique currently available for assessing modulation of multiple PMN functions by pharmacological agents.

52 THE CHEMOTACTIC RESPONSE OF POLYMYXIN-INDUCED GUINEA PIG EOSINOPHILS

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Repeated injections of the mast cell degranulating antibiotic polymyxin B into the peritoneal cavity of guinea pigs elicit an exudate containing 40-60% eosinophils (Blood 52 : 127, 1978). The chemotactic migration of these cells was analyzed in Boyden chambers using cellulose nitrate filters of 3 µm pore size and the leading front method for evaluation after a migration period of 2 h. With the eosinophils suspended in Hanks' balanced salt solution (HBSS) containing 10% guinea pig serum or 0.5% bovine serum albumin, there was no attraction by any of the following substances: yeast-activated guinea pig serum (which was strongly chemotactic for glycogen-induced guinea pig neutrophils), the ECF-A tetrapeptides Ala-Gly-Ser-Glu and Val-Gly-Ser-Glu (10^{-4} - 10^{-9}mol/l), histamine (10^{-4} - 10^{-9}mol/l), a combination of the tetrapeptides and histamine, the prostaglandins E_2, $F_{2\alpha}$ and D_2 (10^{-5} - 10^{-8}mol/l), and the neutrophil attracting tripeptide f-Met-Leu-Phe (10^{-5} - 10^{-9}mol/l). Eosinophils were strongly attracted by the supernatant of rat peritoneal cells, containing 7% mast cells, stimulated with 48/80, and the supernatant of guinea pig neutrophils stimulated with ionophor A23187 (0.5 µg/ml). The two eosinophil chemotactic factors (ECF) coeluted from a Sephadex G-25 column. They were not destroyed by 2 multispecific peptidases. When added to the cells in the upper compartment of the Boyden chamber both ECF preparations inhibited the migration of the eosinophils towards the identical as well as the other ECF. After a 10 min 37°C incubation of the cells with ECF at concentrations used for chemotaxis and subsequent washing, the cells were not inhibited in their migration to either ECF. These unusual findings suggest that eosinophils elicited by different methods differ in their chemotactic response to various agents.

53 EVIDENCE OF A QUARTZ-INDUCED CHEMOTACTIC FACTOR FOR GUINEA PIG ALVEOLAR MACROPHAGES.

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Broncho-pulmonary cell populations were obtained from guinea pigs exposed to quartz dust by inhalation. Extracts derived from the disrupted cell populations were chemotactic for resident guinea pig alveolar macrophages. Similar chemoattractant activity was not however, demonstrated in extracts of broncho-pulmonary cell populations obtained from control animals. Resident alveolar macrophages also consistently migrated in response to supernatants obtained

from alveolar macrophage cultures of guinea pigs exposed to quartz, and cultured for either 4 or 18 hours. The chemotactic activity was found in supernatants from these cultures whether guinea pigs had been exposed for 3 months or exposed for 3 months and then maintained under normal animal house conditions for a further 3 months. These quartz-induced chemoattractants may account for the recruitment of mononuclear phagocytes into the lung during the evolution of silicotic lesions.

INFLUENCE OF VARIOUS ANTIBIOTICS ON POLYMORPHONUCLEAR CHEMOTAXIS AND RANDOM MIGRATION **54**

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Polymorphonuclear granulocytes play an important role in the immediate unspecific host response and a depression of their functions can be found in many patients with severe or recurrent infections. Therefore administration of drugs causing such impairement in PMN functions may be regarded as an additional risk for negative side effects to the patient. In the present work the effect of 13 antibiotics -amphotericin B, amoicillin tauglicolcillin, amoxocillin, cloxacillin, cefaloridine, cefalexin, cefuroxime, chloramphenicol, gentamicin, rifamycin fosfomycin- on the granulocyte spontaneous and induced migration was investigated under in vitro experimental conditions. Human PMN preincubated with the antibiotics approprioately brought to the concentrations desired (therapeutic dose, 1/10 and 10 X) in hepes-medium 199-water solution pH 7.2, were washed 3 times and tested for spontaneous and induced migration under agarose. Our experiments demonstrate that amphotericin B, cefalexin, cefaloridine, cefuroxime, chloramphenicol, dicloxacillin, gentamicin and rifamycin can inhibit in vitro human PMN chemotaxis and/or random migration. Inhibition of intracellular respiratory enzime synthesis, presence of inactive metabolites of the drug, alterations of cyclic AMP and GMP or of the divalent cations which are membrane bound can be responsible of the phenomenon.

The following abstracts were received after the closing deadline for presentation on Wednesday 30 July.

LYMPHOKINE/TRANSFER FACTOR

PREPARATION, PROPERTIES, AND CLINICAL USE OF HUMAN TRANSFER FACTOR AND "IMMUNE" RNA **55**

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Transfer factor (TF) and "immune" RNA (iRNA) were prepared from normal peripheral blood leucocytes disintegrated in cold 40% ethanol. The supernatant fluid after removing the alcohol was then passed through an ultrafiltration membrane to obtain TF. The residue was used to extract the "iRNA" with SDS-phenol. This method is simple, avoids contamination with bacteria and is suitable for large scale production of both TF and "iRNA." Each unit of TF is equivalent to 0.5gm leucocytes containing 6.86 $\pm$ 0.72mg dry substance, 182 $\pm$ 26 ug ribose, 797 $\pm$ 197ug polypeptides, and 58 $\pm$ 10ng CAMP. $E_{250} = 7.86 \pm 0.98$, $E260/E_{280} = 2.62 \pm 0.12$. The UV absorption spectrum gave a peak at 250-251nm. TLC revealed at least four spots stained with ninhydrin. Sephadex G-25 filtration gave a characteristic elution pattern yielding a major peak near the total volume (Vt) of the column and several smaller peaks beyond the Vt. The iRNA preparations contained RNA 48 $\pm$ 4%, DNA 4 $\pm$ 2%, Protein 2 $\pm$ .3% and glycogen 42 $\pm$ 10%. $E_{260}/E_{280} \geq 2$, at least five bands were revealed in 3% PAGE corresponding to 4S, 8S, 9S, 15S, and 20S RNAs. The "iRNA" preparation possessed the ability to restore E-rosette formation of heated peripheral blood lymphocytes. The animal experiments demonstrated both the TF and the "iRNA" preparation were nontoxic, nonpyrogenic, and non-anaphylactic. So far, over 700 patients afflicted with various viral and fungal infections, autoimmune diseases, and malignant diseases were treated with TF and over 100 patients were treated with "iRNA". After injections, the percentage of OT and SK-SD skin tests which converted from negative to positive were 27.1% and 42.9%, respectively with TF and 78.4% and