partly for the maximum peak of blood flow attained in homografts; and (d) the increase of mediators after the onset of rejection is the result of non-specific tissue breakdown.

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Lysosomal enzyme release from leucocytes by N-formyl-L-methionyl-L-leucyl-L-phenylalanine *in vitro:* effect of some anti-inflammatory drugs

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When rabbit peritoneal leucocytes are incubated, *in vitro*, with low concentrations of N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP) in the presence of cytochalasin B (5μg/ml), β-glucuronidase (a lysosomal marker enzyme) is released (Becker, 1976). Neither FMLP nor cytochalasin B alone induce β-glucuronidase release. This process is not accompanied by release of the cytoplasmic marker enzyme lactate dehydrogenase (LDH) (Becker, 1976). We have studied the effect of some anti-inflammatory drugs on the process.

Human leucocytes were exposed to cytochalasin B and drug for 15 min before the addition of FMLP. All agents were dissolved in dimethylsulphoxide (DMSO) to give a final concentration of DMSO of 0.75% in a saline medium. After 15 min incubation at 37°C the leucocytes were centrifuged at 12,000*g* for 30s and the supernatant removed for enzyme assay. β-Glucuronidase was measured by the method of Ringrose, Parr & McLaren (1975), and LDH by the method of Wroblewski & LaDue (1955). Total enzyme present in the leucocytes was determined by addition of Triton X-100 to give a final concentration of 0.2%. Controls were included to determine basal enzyme release as well as any effects of the drugs on the activity of β-glucuronidase and LDH.

The log dose-response curve for release of β-glucuronidase by FMLP in the presence of cytochalasin B (5μg/ml) was sigmoid, as described by Becker (1976) for rabbit peritoneal granulocytes. The EC50 in our conditions (2.3×10^{-8}M) was about one hundredfold greater. Human cells are similarly less sensitive to FMLP as chemotactic stimulant (Williams, Snyderman, Pike & Lefkowitz, 1977).

Release of enzyme was inhibited by hydrocortisone (10^{-3}M). At this concentration of hydrocortisone, FMLP, even at 10^{-4}M, released only about 5% of the control amount; whereas at 10^{-4}M hydrocortisone, release was barely inhibited. The antagonism therefore appeared to be non-competitive or insurmountable.

Indomethacin and phenylbutazone also inhibited release, moving the dose response curve to the right without loss of parellelism, and giving pA_{10} of about 4.05 and 4.96 respectively. Aspirin was inactive at 10^{-3}M but papaverine (5×10^{-5}M) and theophylline (10^{-3}M) were inhibitory. Neither LDH release nor the activity of β-glucuronidase was affected at any of these concentrations of drug.

These results suggest that treatment of leucocytes with FMLP and cytochalasin B activated an enzyme, which was competitively inhibited by indomethacin and phenylbutazone. If the enzyme were involved in prostaglandin synthesis, the effect of hydrocortisone could be attributed to inhibition of release of prostaglandin precursors, and that of inhibitors of phosphodiesterase to increase of tissue cyclic AMP.

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Mechanisms of collagen-induced bronchoconstriction and thrombocytopenia in the guinea-pig

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Circulating platelets are not required for bronchoconstriction due to the thromboxane A2 precursor arachidonic acid (AA), but are needed for bronchoconstriction by ADP and ATP (Lefort & Vargaftig, 1975). We have now investigated the correlation between bronchoconstriction (Holmes, 1977) and thrombocytopenia due to the standard platelet aggregating agent collagen (Horn Chemical, Munich). Pentobarbitone anaesthetized guinea-pigs were prepared for recording of pulmonary resistance (bronchoconstriction) to inflation. All injections were given intravenously. Platelets were counted from arterial blood automatically, and *in vitro* platelet aggregation was studied by the turbidimetric technique in citrated platelet-rich plasma. Collagen (50–300 μg/kg) induced dose-related bronchoconstriction accompanied by thrombocytopenia, with a peak of 60 ± 21 (% drop in platelet counts ± s.d.) within 3 min for 300 μg/kg. Higher amounts of collagen killed the animals from irreversible bronchoconstriction and hypotension. Aspirin and indomethacin (1 and 5 mg/kg respectively) inhibited bronchoconstriction, but caused a statistically insignificant reduction in thrombocytopenia. Drugs which failed to interfere with the effects of collagen were (mg/kg): cyproheptadine (0.5), atropine (1), mepyramine (2), soybean trypsin inhibitor (20), carboxypeptidase (5, enough to suppress bronchoconstriction by bradykinin), ADP (infused at 1 mg kg^{-1} h^{-1}, enough to inhibit bronchoconstriction and thrombocytopenia by ATP and by ADP itself), and the Künitz trypsin inhibitor (100,000 u/kg). This ruled out a role for histamine, serotonin, acetylcholine and bradykinin in collagen-induced bronchoconstriction. Failure of platelet desensitization with ADP to inhibit bronchoconstriction suggested that platelets are not involved directly, but platelet antiplasma (Vargaftig & Lefort, 1977) suppressed bronchoconstriction. Guinea-pig platelets are aggregated by complement-derived peptides (Benner, Schumacher & Glassen, 1975; Grossklaus, Damerau, Lemgo & Vogt, 1976), and the complement component Clq is a collagen-like glycoprotein (Müller-Eberhard, 1975). Since immune platelet depletion presumably is complement-dependent, complement activation was thought of as a mechanism of action of collagen *in volvo*. The complement-depleting agent carrageenin (Davis, 1965), infused for 30 min at 1 mg/kg, prevented bronchoconstriction and thrombocytopenia by collagen, but not by AA, and failed to block thrombocytopenia or *in vitro* aggregation of platelets collected at the end of the infusion. A platelet site of action for collagen-induced bronchoconstriction is suggested, but complement-derived peptides may be involved also, and trigger generation of thromboxane A2, thus explaining effectiveness of aspirin and of indomethacin.

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