

From Institute of Pathophysiology and Institute of Microbiology,
University Medical School, Debrecen, Hungary.

MACROPHAGE DISAPPEARANCE REACTION INDUCED BY LYMPHOKINES

By

S. SIPKA, I. BOLDOGH & T. SZILÁGYI

Lymphokines, the biological active products of lymphoid cells responding to antigen or mitogens are considered to be potential mediators of cellular immunity (11). Their role is manifested in delayed-type hypersensitivity reactions, in protective immunity and in cell cooperation effects (4).

Nelson & Boyden (9) have shown that the intraperitoneal injection of antigen into delayed hypersensitive guinea pigs bearing glycogen-induced peritoneal exudates leads to a marked reduction in the macrophage content of these exudates. This "macrophage disappearance reaction" (MDR) was regarded as a manifestation and an *in vivo* model of delayed hypersensitivity (8, 9, 10). The reason for the "disappearance" of macrophages is their adherence to the lining of the peritoneal cavity (10).

Jókay & Karcagh (5) elucidated important details in the pathomechanism of MDR. They were able to induce MDR by intraperitoneal injection of thrombin in normal mice suggesting the participation of a coagulation system in the phenomenon.

Sonozaki & Cohen (12) considered that the MDR was mediated by a soluble lymphocyte-derived factor, by a lymphokine or lymphokines.

Maillard et al. (7) reported that the supernatants of lymphocytes from sensitized guinea pigs (showing lymphokine activity) had slight procoagulant activity. Recently, Coeugnet

& Bendixen (1) proved that lymphokines derived from concanavalin A (Con A)-stimulated human lymphocytes had a thrombin-like procoagulant activity.

In our experiments we induced MDR in normal (non-sensitized) guinea pigs by a) human lymphokines deriving from Con A-stimulated lymphocytes, b) ellagic acid (an activator of intrinsic coagulation) and c) thromboplastin (an activator of extrinsic coagulation).

MATERIAL AND METHODS

Animals. Albino guinea pigs of both sexes weighing 300–400 g were used in MDR. The animals were kept under standard conditions, fed on standard diet and allowed to drink water *ad libitum*.

Materials. Concanavalin A (Sigma), ellagic acid (Koch-Light), thromboplastin liquid (Hyland).

Preparation of lymphokines. Normal human blood anticoagulated by citrate was used. The preparation of lymphocytes was carried out in Uromiro-Ficoll solution in Parker's 199 medium at room temperature. No serum was added. The samples were centrifuged at 800 *g* for 15 min and washed twice; they contained 90–95 per cent mononuclear cells.

10^7 Lymphocytes were cultured at 37° C in 5 per cent carbon dioxide and 95 per cent air milieu in the presence of 20 μ g Con A completed with 100 iu/ml penicillin and streptomycin for 36 h. The cell-free supernatants were chromatographed on Sephadex G-75 column using 0.01 M phosphate buffer pH 7.4 for elution. In the control preparation Con A was added at the end of cultivation. The protein-containing fractions were collected and concentrated with Amicon B-15. The protein concentration of the preparations used was generally about 1 mg/ml.

We named the leukocyte migration inhibitory factor and the skin reactive factor, which contained preparations with procoagulant activity, active supernatants (AS) and the controls (CS). Leukocyte migration inhibitory factor activity was tested with human leukocytes according to Szabó's method (6). Skin-reactive factor testing was carried out in rat skin based on the measurement of the blue areas on the inner surface of the skin after intravenous injection of 1 per cent Evans-blue. Procoagulant activity of the AS was tested for its effect on the recalcification time, partial thromboplastin time and kaolin clotting time of thrombocyte-poor human plasma measured with Clotek (Hyland) equipment.

Induction of MDR. For the induction of macrophage-rich peritoneal fluid normal guinea pigs were injected intraperitoneally with 20 ml of paraffin oil 4 days prior to MDR testing. On the 5th day we injected the animals intraperitoneally with 0.5 ml of lymphokines, 60 mg/kg of ellagic acid dissolved in saline and 1 ml of thromboplastin liquid.

The control animals were administered with the same quantity of control samples of lymphokines, or with the dissolving materials. Four hours after the injection of test materials the animals were bled and their peritoneal cavities were washed out with 40 ml of Parker solution anticoagulated by heparin (5 iu/ml). These procedures were carried out in plastic tubes and dishes. Peritoneal exudate containing macrophages were centrifuged and washed twice with Parker solution. The cells were collected in standard volumes and quantitatively and qualitatively valued. We estimated the whole number of the cells in a Bürker chamber and differential cell countings were carried out by May-Grünwald-Giemsa staining.

RESULTS

Table 1 shows that all the materials tested, i.e. samples of lymphokines, ellagic acid and thromboplastin, induced a remarkable decrease in the number of macrophages in the peritoneal fluid of normal guinea pigs. The lymphokines also caused eosinophilic chemotaxis. They probably contained eosinophilic chemotactic factor (2).

TABLE 1

Induction of Macrophage Disappearance Reaction in Normal Guinea Pigs by Lymphokines, Ellagic Acid and Thromboplastin.

Groups of animals	Cell count/ml in peritoneal fluid, mean $\pm$ s.d.		
	Macrophages	Lymphocytes	Eosinophils
Control of lymphokines (CS)	$1.42 \pm 0.2 \times 10^7$	$0.32 \pm 0.12 \times 10^7$	$0.10 \pm 0.05 \times 10^7$
Lymphokines (AS)	$0.62 \pm 0.2 \times 10^7$	$0.49 \pm 0.2 \times 10^7$	$0.60 \pm 0.2 \times 10^7$
Phys. NaCl	$1.64 \pm 0.3 \times 10^7$	$0.42 \pm 0.10 \times 10^7$	$0.05 \pm 0.01 \times 10^7$
Ellagic acid	$0.55 \pm 0.2 \times 10^7$	$0.40 \pm 0.15 \times 10^7$	$0.05 \pm 0.01 \times 10^7$
Thromboplastin	$0.42 \pm 0.15 \times 10^7$	$0.44 \pm 0.2 \times 10^7$	$0.05 \pm 0.01 \times 10^7$

Averages of three experiments.

DISCUSSION

Our data support the suggestion that the coagulation system plays an important role in the pathomechanism of MDR. The human lymphokines showing procoagulant activity were able to induce MDR in normal non-sensitized guinea pigs. This effect manifested, in spite of the species differences, similarly to other lymphokine actions (13).

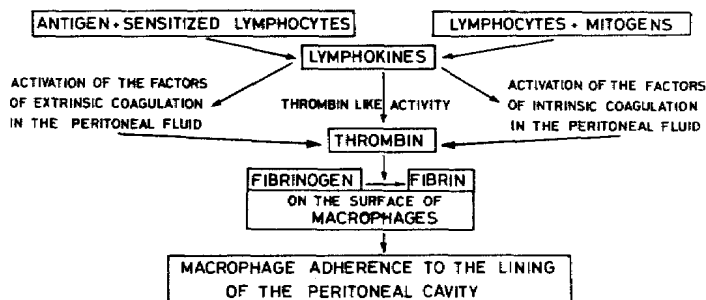


Fig. 1.

Possible pathomechanism of macrophage disappearance reaction.

We regard the procoagulant activity of lymphokines to be the triggering factor of MDR. The lymphokines, due to their thrombin-like activity (1), can directly induce fibrin production from the fibrinogen on the surface of peritoneal macrophages (13). Beside this ability, the peritoneal fluid contains coagulation factors of both extrinsic and intrinsic coagulations and their activation can also result in MDR.

Combining our results with previous data we suggest the following scheme as an explanation of the pathomechanism of MDR (Fig. 1).

SUMMARY

Macrophage disappearance reaction was induced in normal (non-sensitized) guinea pigs by human lymphokines deriving from Con A-stimulated lymphocytes, by ellagic acid (an activator of intrinsic coagulation) and by thromboplastin (an activator of extrinsic coagulation). The results are discussed from the aspect of the pathomechanism of macrophage disappearance reaction.

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Authors' address:

Sándor Sipka
Institute of Pathophysiology
University Medical School
H-4012 Debrecen, Pf. 23
Hungary

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