FAILURE OF GUINEA PIG PLASMA KALLIKREIN TO CAUSE NEUTROPHIL MIGRATION

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Abstract—Guinea pig plasma kallikrein was apparently chemotactic for guinea pig neutrophils when assayed in Boyden's chamber. However, it was concluded that this phenomenon was artificial due to the following reasons: (1) Kallikrein was only chemotactic in the presence of bovine serum albumin (BSA) in the chamber but not when BSA was substituted by guinea pig serum albumin or egg albumin. (2) Kallikrein injection failed to cause dermal tissue leukocytosis. (3) Kallikrein did not polarize neutrophils. A chemoattractant(s) seemed to be generated from BSA preparation time dependently. Although this generation required the enzymatic activity of kallikrein, the chemotactic activity once generated was resistant to a kallikrein inhibitor (diisopropylfluorophosphate) and antiprekallikrein antibody.

INTRODUCTION

Vascular permeability enhancement and neutrophil infiltration are the most prominent phenomena at the early stage of inflammatory process. It was previously reported that guinea pig plasma kallikrein caused vascular permeability enhancement when injected into guinea pig skin, and this reaction was dependent on subsequent kinin generation by kallikrein in vivo (1). Plasma kallikrein has also been studied for its ability to migrate neutrophils. Kaplan et al. demonstrated an appearance of the chemotactic activity for human neutrophils by the conversion of human prekallikrein to kallikrein with activated Hageman factor. This activity was closely linked with the enzymatic activity of kallikrein (2). On the other hand, Wiggins et al. showed that rabbit plasma kallikrein was not directly chemotactic for rabbit neutrophils, but indirectly chemotactic through the release of C5a-like molecule from the fifth component of rabbit complement (C5) (3). Weiss et al. separately reported a diminished generation of kaolin-activable chemotactic activity in prekallikrein deficient serum. How-
ever, this chemotactic defect could be corrected not only by reconstitution with prekallikrein but also by the addition of activated Hageman factor instead of prekallikrein into the serum, suggesting that the absolute contribution of kalli-
krein to chemotactic activity of kaolin-activated serum is small (4). In in vivo experiments using skin abrasions in patients with prekallikrein deficiency, Poon et al. observed the defectiveness of neutrophil infiltration (5), whereas Hathaway et al. reported the infiltration to be within the normal range (6). Hence, the function and contribution of kallikrein to neutrophil migration are still in an uncertain state.

In the present study, guinea pig plasma kallikrein was investigated for chemotactic activity by means of neutrophil polarization assay, Boyden’s chamber assay, and skin injection into guinea pigs given $^{51}$Cr-labeled neutrophils intravenously in advance. These investigations demonstrated the negative activity of plasma kallikrein for the neutrophil chemotaxis.

**MATERIALS AND METHODS**

**Animals** Albino-Hartley strain guinea pigs of both sexes (300–700 g body weight) were used.

**Substances.** All chemicals obtained from commercial sources were of the highest purity grade. Heparin, hexadimethrine bromide (polybrene), diisopropylfluorophosphate (DFP), N-2-hydroxy-
ethylpiperadine-N'-2-ethanesulfonic acid (HEPES), prostaglandin E$_2$, zymosan, and bovine serum albumin (BSA) were obtained from Sigma (St. Louis, Missouri). Egg albumin was purchased from ICN-Nutritional Biochemicals (Cleveland, Ohio). Dextran T 500 was obtained from Pharmacia (Uppsala, Sweden). N-Formyl-methionyl-leucyl-phenylalanine (FMLP), carbobenzyloxy-phenyl alanyl-arginine-4-methylcoumaryl-7-amide (z-Phe-Arg-MCA) were products of Protein Research Foundation (Osaka, Japan). Sodium metrizoate–Ficoll mixture for guinea pig lymphocyte separa-
tion (GP-SMF, code no. 602002) was obtained from Ohtsuka Assay Institute (Tokushima, Japan). $^{51}$Cr in sterilized saline (NEZ-030S) was purchased from New England Nuclear (Boston, Massa-
chusetts). Nucleopore membrane (13 mm, pore size 2.0 μm, N-200, CPC 01300) was purchased from Nomura Micro Science (Tokyo, Japan). All other chemicals were obtained from Wako Pure Chemicals (Osaka) and Nakarai Chemicals (Kyoto, Japan).

**Plasma and Serum.** Blood was collected from guinea pigs by cardiac puncture, mixing nine parts blood and one part anticoagulants [disodium ethylene diamine tetraacetate, dihydrate (1.5 %), glucose (5 %) and polybrene (0.05 %)], and the plasma was obtained by centrifugation according to Yamamoto et al. (7). Zymosan-activated serum was prepared by the method of Fernandez and Hugli (8).

**Assay of Enzymatic Activity of Kallikrein.** The enzymatic activity of kallikrein was measured for the amidolysis using a specific fluorogenic substrate, z-Phe-Arg-MCA, as described by Imamura et al. (1).

**Preparation of Guinea Pig Plasma Kallikrein, High-Molecular-Weight Kininogen, and Al-
bumin.** Plasma kallikrein was purified from guinea pig plasma by successive column chromatography as follows: first DEAE-Sephadex, second DEAE-Sephadex, CM-Sephadex, isoelectric focusing, first Sephadex G-150, and second Sephadex G-150 column chromatography. The purified kallikrein was homogenous when analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Details have been previously reported (1). Guinea pig high-molecular-weight kininogen (HMWK) was prepared from guinea pig plasma by successive column chromatography as