JOINT FLUID LEUKOCYTE ACTIVATION BY PREFORMED IMMUNE COMPLEXES

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Abstract—Acute synovitis was induced in rabbit knee joints by intraarticular injection of preformed bovine serum albumin (BSA) – anti-BSA immune complexes (ICs). Polymorphonuclear granulocytes (PMNGs) which had migrated into joints injected with ICs were degranulated and contained ICs as revealed by electron microscopy and were activated as revealed by the measurement of chemiluminescence (CL). In contrast, leukocytes from control joints injected with BSA and normal rabbit serum as well as glycogen-elicited peritoneal leukocytes did not display any morphological changes and did not show CL. Compared to cells from other sources, leukocytes from IC joints showed a decreased CL response when stimulated in vitro with ICs but not with opsonized zymosan, suggesting a stimulus-specific modification of the PMNG responsiveness. Inhibition experiments showed that oxygen radicals and formation of arachidonate metabolites, mainly of the lipoxigenase pathway, were involved in the CL response of the IC-stimulated joint fluid PMNGs. Our observations on morphology, activity, and responsiveness of emigrated cells from the various sources suggest, together with previous observations, that the reaction of leukocytes in IC-induced synovitis consists of an initial migration phase not related to an increased CL and a subsequent activation phase characterized by degranulation, phagocytosis of ICs and increased CL.

INTRODUCTION

Immune complexes (ICs) have been implicated as important pathogenetic factors in different human inflammatory diseases, e.g., rheumatoid arthritis (RA) (1). Leukocytes accumulate in RA joint fluid (2), and display morphological signs of activation (3). Further, ICs are detected in joints of patients with RA (4, 5) and RA joint fluid induces chemiluminescence (CL) in peripheral blood polymorphonuclear granulocytes (PMNGs) (6).

ICs interact with membrane receptors of macrophages and PMNGs. The binding of ICs to leukocytes initiates a burst of oxidative metabolism, generation of oxygen-derived products (7), endocytosis, and release of lysosomal enzymes (8, 9). PMNGs also emit light during the oxidative burst and, although...
the precise mechanism is not known, this CL has been useful as a measure of peripheral blood leukocyte activation (10). ICs activate human (11–13) and guinea pig (14) blood PMNGs as observed by CL. The induced CL is inhibited by agents interfering with the arachidonate metabolism and oxygen radical generation (13). As RA joint fluids induce a similar CL (6), this suggests that ICs in joints can affect several metabolic pathways in PMNGs leading to the formation of products of importance for the development of synovitis.

Little is known about the characteristics of the injurious ICs in arthritis, about the interaction between these ICs and leukocytes during different phases of the inflammatory reaction, as well as about the products released in the joint by the activated leukocytes. We have previously demonstrated that leukocytes accumulate with different kinetics in the rabbit knee joint after intraarticular injection of preformed ICs of different composition (15). These joint fluid leukocytes aggregate, adhere to the focally eroded synovial lining surface, and phagocytose ICs (16).

In the present study we have used chemiluminescence and electron microscopy to further examine the activation of PMNGs by ICs in this in vivo experimental model.

**MATERIALS AND METHODS**

**Induction of Acute Synovitis**

*Animals.* Rabbits of either sex, weighing 2.5-3 kg were used. The animals were anaesthetized with fluanizone (Hynorm Vet, Leo, Helsingborg, Sweden) 0.7 ml/kg body wt, intramuscularly.

*Immune Complexes.* Antiserum to bovine serum albumin (BSA) was produced in rabbits as previously described (15). ICs were prepared in vitro by incubation for 60 min at 37°C of equal volumes of the antiserum and the appropriate concentration of BSA dissolved in saline. ICs were characterized by their precipitation profiles and complement-activating properties (15). The antiserum formed maximum amount of precipitate with 0.32–1.25 mg/ml of BSA, and the highest complement activation in vitro occurred with ICs formed with 0.64–1.25 mg/ml of BSA. The ICs used in the present study were prepared in slight antigen excess by incubating equal volumes of antiserum with 1.6 mg BSA/ml.

*Knee-Joint Injection and Sampling.* In each rabbit, one joint was injected with 0.5 ml of the IC solution and the contralateral (control) joint with 0.5 ml of a mixture (prepared by incubation for 60 min at 37°C) of equal volumes of normal rabbit serum (NRS) and 1.6 mg BSA/ml. The injections were performed as previously described (17).

Four or 6 h after injection, the rabbits were sacrificed by a lethal dose of sodium pentobarbital (Mebumal, ACO, Solna, Sweden) intravenously. The animal was put on its back with the hind leg stretched and fixed in an elevated position. A longitudinal skin incision was made on the ventral aspect of the knee joint and the joint cavity opened by transverse sectioning of the quadriceps tendon distal to the patella. Great care was taken to avoid bleeding into the joint. The synovial fluid was collected by washing, mainly the suprapatellar pouch, ten times with 0.5 ml phenol red-free Hanks’ balanced salt solution (HBSS).