Studies of Synovial and Serum C-Reactive Protein in Experimental Arthritis in Rabbits[1] (36969)

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Inflammation results in the appearance of C-reactive protein (CRP) in man and Cx-reactive protein in rabbit. These serum proteins are entirely analogous physiologically (1) and closely related immunochemically (2). The nominal distinction between them is based on minor differences in the state of the polysaccharides with which they can combine (1). In this communication the single term CRP will be used to refer to this acute phase protein of both man and rabbit.

The mechanisms mediating the appearance of CRP in response to inflammation are not known. Amino acids are incorporated into CRP by liver slices *in vitro* (3). This finding is consistent with hepatic synthesis of either native CRP itself or, alternatively, a CRP precursor. The latter alternative is supported by a prevous report suggesting that inflammation results in conversion of a preformed precursor into detectable rabbit CRP (4).

If the effect of inflammation is indeed to convert a precursor into detectable CRP, as hypothesized, such conversion might occur at the inflammatory site. Such a mechanism has been proposed (5), and could account for the immunohistologic localization of rabbit CRP in sites of inflammation but not in liver (6). In addition, this mechanism would explain the finding of CRP in synovial fluid, but not in serum, of some patients with various arthritides (7), as well as the evidence suggesting local production of CRP in rabbit aqueous following induction of uveitis (8). However, the report that CRP appears in blood before appearing in blister fluid in human burns is inconsistent with this thesis (9), suggesting that CRP is not formed at the inflammatory site. To help resolve this question, the time course of appearance of CRP was determined in serum and synovial fluid following experimental induction of arthritis in rabbits.

Methods. Mild arthritis of the knee was produced in 17 rabbits by the intra-articular injection of 0.5 ml of a 1% solution of human fraction II,[2] heat aggregated at 65° for 20 min. Severe synovitis was induced in 13 other rabbits by intra-articular injection of 1 ml of 2% croton oil in corn oil. Blood was obtained from the marginal ear vein before the rabbits were sacrificed at intervals of 4 to 48 hr after injection. Following sacrifice, the knee joint was opened and synovial fluid was removed with a Pasteur pipette. Fluid found to be bloody was not studied. Cells and oil were removed by centrifugation in capillary tubes. Synovial and serum samples were stored at −20°.

Serum and synovial concentrations of CRP were determined by the radial immunodiffusion technique previously described (10, 11). This method is able to detect as little as 0.3 μg N CRP/ml with accuracy. Synovial fluid was treated with hyaluronidase,[3] which did not alter diffusion behavior of CRP as estimated by the double diffusion method of Allison and Humphrey (12). Similarly, this method revealed no difference in diffusion rate between synovial and serum CRP. An acute phase rabbit serum whose CRP concentration had been determined by classical quantitative precipitin techniques (13) was employed as a reference standard.

Results. The mild arthritis induced by intra-articular injection of heat-aggregated hu-

[1] This study was supported by U.S. Public Health Service Grant AI 08366, and NIH General Research Support Grant FR 5410.

[2] E. R. Squibb, New York, NY.

[3] Worthington Biochem. Corp., Freehold, NJ.

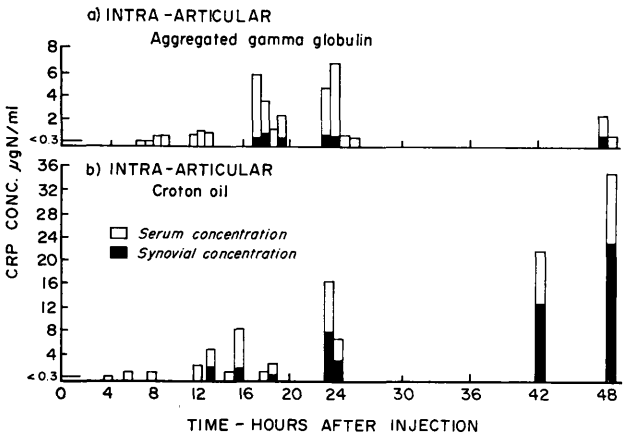

FIG. 1. Serum and synovial CRP concentrations in rabbits with experimentally induced acute arthritis. Each bar represents an experiment in a single rabbit. When synovial CRP was undetectable, a white bar only is shown. Undetectable serum CRP is indicated by a white bar to the level of "< 0.3 μg N/ml," the lower limit of sensitivity of the method. Note the difference in scale on the ordinates in (a) and (b).

man gamma globulin appeared to abate after approximately 24 hr. Serum CRP concentrations were consistent with these observations, reaching maximal values 24 hr after injection (Fig. 1a). CRP could not be detected in synovial fluid until 18 hr after intra-articular injection, although it was found in serum as early as 8 hr after injection.

Intra-articular injection of croton oil in rabbits produced an intense inflammatory reaction which increased in severity for at least 48 hr. Concentrations of CRP in serum samples obtained at the time of sacrifice generally increased with time (Fig. 1b). Maximum serum CRP concentrations were much greater than those observed following injection of aggregated gamma globulin. Although CRP could be detected in serum as early as 6 hr following induction of arthritis, synovial CRP was not detected earlier than 12 to 15 hr after injection. In neither group of rabbits was CRP ever detected in synovial fluid prior to its appearance in blood, nor did synovial concentration ever exceed serum concentration.

Discussion. Synovial and serum CRP concentrations were studied in rabbits with induced mild and severe arthritis to test the possibility that formation of CRP from precursors occurs at the inflammatory site. These studies revealed that CRP always appeared in synovial fluid later and at lower concentrations than in serum. In order to confirm applicability of the results of this study to man, CRP concentrations were determined in specimens of synovial fluid and serum obtained simultaneously, from 25 patients with various arthritides. Synovial concentrations of CRP did not exceed levels anticipated for a protein of its molecular size (10), and were never greater than serum concentration in these patients; CRP was never found in synovial fluid when absent from blood. These findings in rabbit and man make the possibility of CRP formation at the inflammatory site extremely unlikely, and indicate that this protein in synovial fluid is derived from serum, its presence reflecting passage from the blood stream.

Failure to find evidence of CRP formation at the inflammatory site indicates that humoral factors, originating at the site of inflammation, must be responsible either directly or indirectly for appearance of circulating CRP following a local inflammatory stimulus. The nature of such presumed blood-borne substances is unknown. A seromucoid substance demonstrated in acute phase rabbit serum might represent such a humoral factor (14), or CRP itself might induce further CRP formation (15). Such circulating factors might induce *de novo* hepatic synthesis, or alterna-

tively, might act by converting precursors into detectable CRP or by protecting newly synthesized CRP from rapid degradation by normally occurring antagonists.

Summary. Serum and synovial C-reactive protein concentrations were determined in rabbits with induced arthritis to test the hypothesis that this acute phase protein is formed from precursors at sites of inflammation. CRP always appeared later and in lower concentration in synovial fluid than in serum. Similarly, in patients with a variety of arthritides, synovial CRP concentration never exceeded values expected as a result of simple passage from blood into synovial space. These findings indicated that CRP is not formed at the inflammatory site. Rather, its formation must occur at sites distant from the site of inflammation, and hence depend on humoral factors.

We are indebted to Dr. James D. Knoke for statistical analysis, to Mrs. Helen Morrison for secretarial help, and to Miss Gail Patek for technical assistance.

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Received Sept. 22, 1972. P.S.E.B.M., 1973, Vol. 142.