KAWASAKI MED. J. VOL. 11 : NO. 3, 187-190 (1985)

Brief Note

A Trial Method for Immune Complex Arteritis under Gelatin Sponge Emboli

Accepted for Publication on August 14, 1985

Key words : Experimental arteritis — Immune complex — Gelatin sponge emboli — Horseradish peroxidase

As a model of allergic cutaneous vasculitis, we have studied Arthus reactions using horseradish peroxidase (HRP) as antigen.[1-4] In these studies, immune complexes were found to be deposited on capillaries to venules of the skin and were phagocytosed by neutrophils. They did not, however, locate on arterial walls. Clinically, arteritis must play an important role to cause ischemic tissue damage in collagen diseases. Some conditions, for instance emboli, may be necessary for the deposition of immune complexes on arterial walls. In order to study the mechanism of arteritis caused by immune complexes, an active Arthus type reaction was induced on a rabbit's auricular artery under a condition of embolization with gelatin sponge (Spongel®) powder.

An albino rabbit was sensitized by injection of 6 mg HRP (type 6, Sigma) suspended with 0.3 ml Freund's incomplete adjuvant, and then boosted with 3 mg HRP after two and four weeks. Five weeks after sensitization the animal was checked by intracutaneous injection of 0.05 mg HRP, and a positive erythematous reaction was confirmed.

Gelatin sponge was pulverized with a file and saturated with HRP dissolved in phosphate buffered saline (pH 7.2). The sponge swelled immediately and while it could pass through a 22 gauge needle, it could not pass through needles of under 25 gauge.

An active Arthus type reaction was induced in the sensitized rabbit by injection of 1 mg HRP dissolved in 1 ml phosphate buffered saline with 20 mg of gelatin sponge powder into the auricular artery through the skin.

Immediately, ischemic change became visible on the artery itself and on the supplied skin from the distal side of the intraarterial injection. Several minutes later the venous plexus dilated and became congested with blood, but the embolized artery remained ischemic and palpably hard. A biopsy specimen was taken from this artery after 26 hours, embedded in paraffin and stained with hematoxylin-eosin.

Under a light microscope, the arterial lumen was found to be occupied by gelatin sponge, which was well stained with hematoxylin and showed a cavernous structure containing erythrocytes and neutrophils. Many neutrophils had infiltrated the arterial wall and the areas around it, especially the adventitia and the junction area between the inner and middle coats. Interestingly, they arranged themselves in a single layer along the internal elastic membrane (Fig. 1).

A control study was done with normal rabbits in the same manner. Arteries were embolized by 1) gelatin sponge with or without HRP, or 2) gelatin sponge

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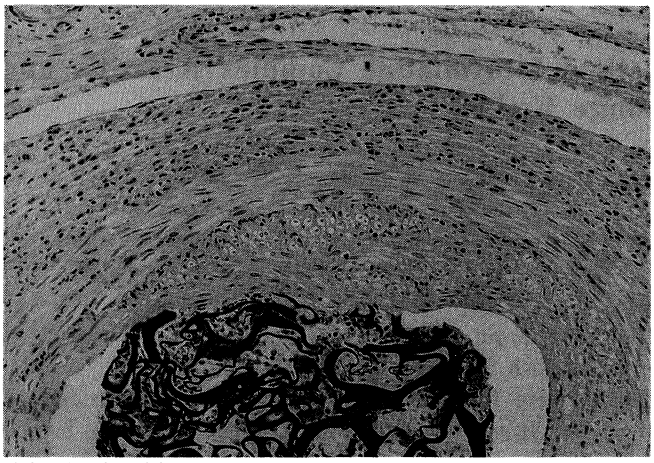

Fig. 1. Sensitized rabbit's artery 26 hours after intraarterial injection of gelatin sponge with HRP. The lumen was occupied by gelatin sponge showing a cavernous structure. Many neutrophils infiltrated within and around the wall. HE, ×200.

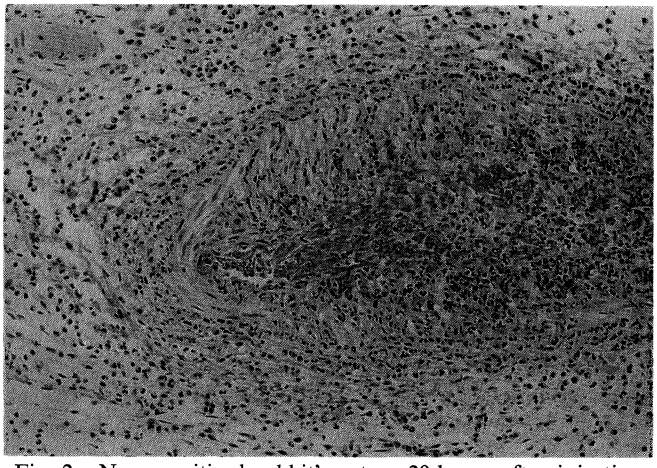

Fig. 2. Non-sensitized rabbit's artery 20 hours after injection of gelatin sponge with in vitro prepared HRP-anti-HRP immune complexes. Neutrophils infiltrated furiously and the structure of gelatin sponge was lost. HE, ×200.

with in vitro prepared HRP-anti-HRP complexes : the sensitized rabbit's serum was conjugated with HRP in moderate antigen excess, and then the sediments were used as insoluble HRP-anti-HRP immune complexes. Biopsies were carried out 20 hours after induction. Severe infiltration of neutrophils was seen in 2) (Fig. 2), and less was seen in 1) (Fig. 3).

In non-sensitized animals, gelatin sponge emboli with or without HRP caused slight neutrophil reactions. On the other hand, severe neutrophil reactions were observed where had been an active Arthus type reaction or an injection of immune complexes. Our previous studies had strongly suggested that the existence of neutrophils in arterial walls was the result of deposition of immune

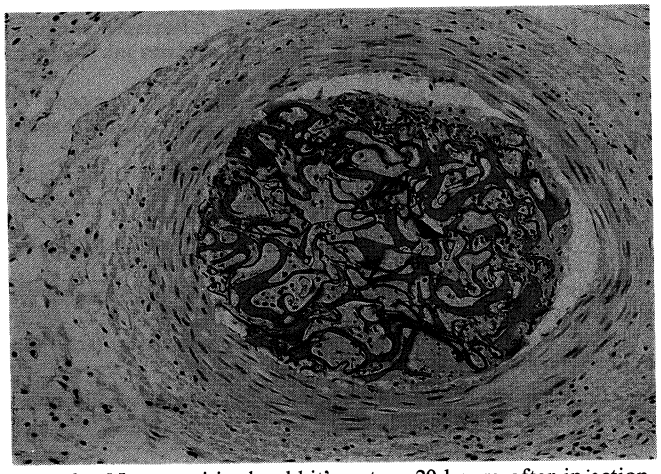

Fig. 3. Non-sensitized rabbit's artery 20 hours after injection of gelatin sponge with HRP. A few neutrophils were seen. HE, ×200.

complexes. Therefore, it is considered that antigen or immune complexes stayed in artery long enough to bring about an immune reaction by means of gelatin sponge embolization.

Spongel® itself has no antigenic qualities,[5] and is absorbed in the tissue within one month.[6,7] Recent clinical and experimental studies have shown that embolization with gelatin sponge does not completely occlude the hepatic artery.[8] Our experience also showed that a proper dosage of emboli into the auricular artery did not cause death of the animal, nor was ischemic necrosis brought on by emboli alone. These characters are convenient to our immunological experiment, moreover a long term follow-up study may be possible.

This trial may provide us with a new and good model for induction of immune complex arteritis. More precise immunopathological investigations of this system are in progress.

Acknowledgment

This work was supported in part by a grant from the Ministry of Education of Japan, Grant No. 60570472 and from a Kawasaki Medical School for Project Research, No. 60-202.

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