chloride solution and injected in a dose of 200 µg in 10 µl into the lateral brain ventricle according to the method described elsewhere[7]. The correctness of the site of injection was checked visually after the killing of each animal. Corresponding control animals received the same volume of the solvent. Additional control animals were injected with tuftsin produced by Serva, in a dose of 200 µg. All solutions injected into the lateral brain ventricle had a pH of 7.4. In a group of 3 rats, naloxone (Endo Lab.) 1 or 2.5 mg/kg i.p. was injected 30 min before TU or Arg-TU. Analgesic activity was measured 140 min after injection (figure). As an additional control, morphine hydrochloride was injected intracerebro-ventricularly (i.c.v.) in a dose of 10 µg. In 5 animals, naloxone at a dose of 1 or 2.5 mg/kg was injected 30 min prior to morphine. 14 rats were used in each group and the results evaluated statistically using Student's t-test.

Both preparations of TU (WU and Serva) elicited marked analgesia during the 30 min immediately after injection. Arg-TU had a more potent analgesic effect, and it persisted for 140 min (figure). Naloxone did not prevent TU or Arg-TU elicited analgesia.

Morphine produced a maximal increase in latency time to 30 sec which persisted for 2 h. This effect was antagonized completely by both doses of naloxone. Arg-TU evoked abnormal behavior in that 5 min after injection of Arg-TU, rats were in recumbent position with stretched limbs. 10 min after injection, barrel rotations occurred (repeated rotation around the animal's longitudinal axis); this lasted for 30 min. 40 min after injection, clonic convulsions were observed which disappeared after 20 min. This abnormal behavior was not antagonized by naloxone. 1 h after injection, the behavior of rats injected with Arg-TU was not different from the behavior of rats injected with saline solution although analgesic activity was still present. The Serva tuftsin appeared to be less, but not significantly less, potent than the WU tuftsin. Serva tuftsin was used as a control for WU tuftsin synthesized by us. The results presented indicate that TU and Arg-TU have an evident effect on the central nervous system. The barrel rotations that appeared after Arg-TU were similar to the phenomenon described after injection of arginine-vasopressin in rats[8]. The absence of an antagonistic effect of naloxone on TU or Arg-TU analgesia suggests that these substances do not act on opiate receptors. Therefore, 2 suggestions can be made: a) Tuftsin and its analogues may represent a new class of substance of interest in the search for new analgesics. b) It may be speculated that there is a link between immunobiological responses and the central nervous system, since there is the possibility that TU continuously synthesised for stimulation of phagocytes could affect the central nervous system.

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Granuloma formation in rat liver after hepatic vein ligation

K. Weinbren, S. Mason and S. Sen

Royal Postgraduate Medical School, DuCane Road, London W12 OHS (England), 21 April 1980

Summary. After occlusion of the hepatic vein draining 1 lobe of the rat liver, macrophage granulomas develop which are reproducible and apparently related to a heat-labile macrophage mobilising factor.

The development of a granulomatous reaction has been described in several different circumstances, some of which involve localization of known antigens of low diffusibility[1], some represent reactions to metallic[2] and other[3] compounds and some relate to familiar pathogens[4]. Hypersensitivity is associated with granuloma formation in many instances but the exact mechanism has not been satisfactorily determined[5]. Often an irritant is responsible for the formation of the granulomatous reaction and the development and resorption of the lesion are to some extent dependent on the potency and ineradicability of the irritant[6].

We wish to report the regular development of macrophage granulomas in rat liver lobes subjected to occlusion of the hepatic veins and with no exposure to known toxins, bacterial pathogens or deliberately introduced antigens.

Methods. Male Wistar rats were maintained on 'Research' rat cubes (Parkes 41B diet) and subjected to surgical procedures at 150-300 g b.wt. All operations were performed under ether anaesthesia and the animals were not fasted preoperatively or before death.

1. The hepatic vein draining the left lobe of the liver was ligated in 95 rats and the animals killed and the livers examined at 24 h intervals up to 15 days and then at longer intervals to 12 weeks.

2. To effect necrosis of part of a liver lobe, the hepatic artery and portal vein to the medial half of the median lobe (which normally has 2 branches of the portal vein and 2 branches of the hepatic artery supplying it) were ligated in 8 rats which were killed between 8 and 10 days.

3. A fragment of fresh omental lobe (5×2 mm) was implanted into the left lobe through a 5 mm capsular incision in 10 rats. These were killed at 8 days.

4. In order to implant necrosed autologous tissue, the omental lobe was infarcted by afferent vascular ligation 21 days before implantation in 6 rats, 14 days before implantation in 6 rats and 7 days before implantation in 6 rats. All animals bearing implants of tissue which had undergone prior in vivo necrosis were killed at 8 days after implantation.

5. Implantation was carried out of omental lobe tissue which had been subjected to immersion in water at 100 °C for 2 min in 6 rats and in water at 60 °C for 2 min in 6 rats. All animals were killed at 8 days. All tissues were fixed in

10% formalin in 80% alcohol, paraffin embedded and stained with haematoxylin and eosin.

Results and discussion. After ligation of the hepatic vein draining the left lobe of the liver, striking changes were noted. After 1 day conspicuous congestion was present, after 2 days obvious necrosis of hepatocyte plates with survival of periportal cells, by 4 days mononuclear phagocytes were abundant and granulomas with epithelioid cells sometimes surrounding eosinophilic necrotic cores were regularly found at 7 days (figure 1). The granulomas increased in numbers with many polykaryons (both Langhans and foreign body type) until 21 days when the granulomas had for the most part contracted and often showed no evidence of the previous necrosis (figure 2). There were conspicuous changes in weight of the ligated lobe with initial enlargement and a subsequent reduction to a thin sliver of tissue.

The possibility that the formation of granulomas might be mediated by the accumulation of lipid-rich erythrocyte membranes or products therefrom was considered as it was known from previous experiments that whole lobar necrosis resulting from afferent vascular occlusion is not associated with the development of granulomas[7]. A further difference between the lesion resulting from hepatic venous occlusion and that caused by afferent vessel (hepatic arterial and portal venous) occlusion was that in the former foci of necrotic liver tissue are juxtaposed to foci of surviving periportal tissue whereas in the latter, the whole lobe is infarcted. An attempt was made therefore to induce necrosis of part of a liver lobe, leaving adjacent liver uninfarcted by ligating the hepatic artery and portal vein to the medial half of the median lobe. After 8 days, palisaded macrophages and granulomas were found in all animals at the junction of infarcted and normal hepatic tissue. This finding indicated that the entrapment of erythrocytes a constant feature of hepatic venous occlusion is unnecessary for the development of granulomas.

It was clear that the necrosed or necrosing liver tissue stimulated the formation of the granulomas and this was confirmed by granuloma formation at 8 days after implantation of a fragment of autologous liver tissue into the left lateral lobes of 10 rats. No such reaction developed if the tissue to be implanted was allowed to necrose in vivo by vascular ligation 21 days previously, but some reaction occurred after 7 days in vivo necrosis though distinctly less than that which occurred with a fresh autologous implant and even less occurred with an implant which had undergone 14 days of prior in vivo anoxic necrosis.

Similarly, no reaction occurred if the implant was placed in water at 100 °C for 2 min and then implanted, but some granulomatous reaction was noted if the implant was subjected to 60 °C for 2 min.

It is of some interest that macrophage granulomas examined in livers subjected to relatively long-term (3 weeks or more) venous ligation frequently contained no residual necrotic tissue and thus resembled hepatic granulomas which are not usually associated with necrosis. The pathogenesis was evident only on examination of early stages in the development of the lesion. The regulatory mechanisms are not clear and although it is likely, it is not certain whether the majority of cells multiply locally; some may be derived from monocytes in the circulation. The present findings suggest that necrosing liver tissue releases a macrophage mobilizing factor which is destroyed by the complete denaturation of 21 days of anoxic necrosis and is inactivated by boiling for 2 min but is partially stable to heating at 60 °C for 2 min. Further studies are necessary to elucidate whether other components participate in the response[8], but necrosing liver tissue seems to be the site of origin. The response was found in all animals subjected to the venous ligation; the granulomas are not contaminated with variable quantities of residual chemical agents used for inducing the inflammatory reaction which may complicate preparations used as a bioassay for putative anti-inflammatory compounds, particularly those designed to inhibit proliferation of macrophages[9].

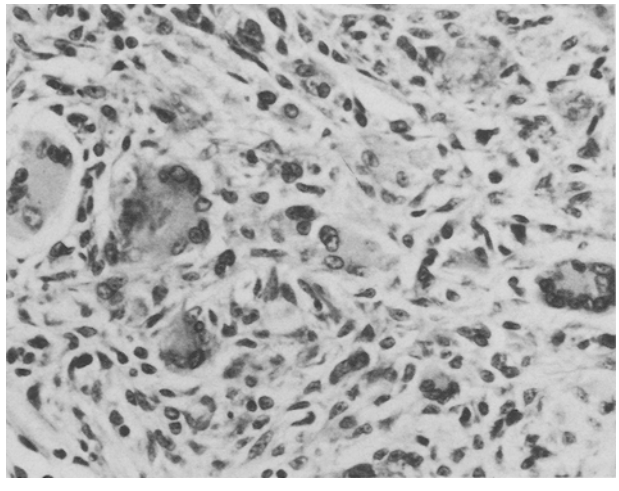

Fig. 1. Giant cell granulomas in left lateral lobe of liver 7 days after hepatic venous ligation. HE × 320.

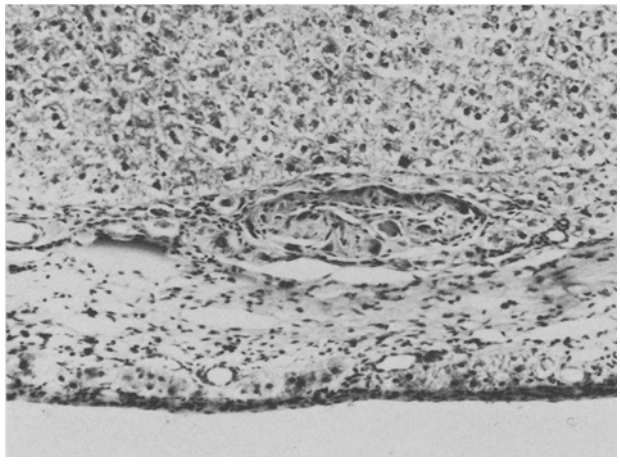

Fig. 2. Fibrosis and productive inflammatory reaction in left lateral lobe of rat liver 30 days after hepatic venous ligation. The normal median lobe is seen below. HE × 120.

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