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Quantitation of Hepatic Sinusoidal Macrophages during the Acute Systemic Inflammatory Reaction in Two Animal Species (42491)

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Abstract. The participation of hepatic sinusoidal macrophages (HSM) in hepatocyte stimulation during the acute systemic inflammatory reaction has been suggested by recent *in vitro* investigations. A first attempt in studying the role of these cells *in vivo* would appear to be the quantitation of HSM at the different times of the inflammatory response, in order to determine whether the participation of HSM depends on the recruitment of blood monocytes to the liver or on the proliferation of resident cells. HSM were counted during the initial stages (0, 16, and 24 hr) of a turpentine-induced inflammation in the rat and the rabbit. They were identified on morphological grounds and were counted separately in the periportal and the perivenous areas of the hepatic lobule. No significant differences were found in the number of HSM per field at 0, 16, and 24 hr following the induction of inflammation. No variation in the distribution of these cells within the lobule could be detected during this period. These results do not support the hypothesis that the acute phase reaction is accompanied by an influx into the liver of newly recruited macrophages or by the proliferation of resident cells. Thus, if a commitment of HSM occurs *in vivo* during the acute systemic inflammation, it may depend on the activation of resident cells.

The acute systemic inflammatory reaction is accompanied by a dramatic increase in the concentration of certain plasma proteins, known as acute phase reactants (APR) (1, 2). Like most plasma proteins, the APR are synthesized by the hepatocytes (3). The rise in their plasma concentrations during the acute inflammatory reaction results from an increased hepatic synthesis (1). Certain factors released by activated cells of the monocyte–macrophage lineage are recognized as the major mediators of hepatic stimulation (4, 5). These probably comprise a family of closely related proteins described under different names: leukocytic endogenous mediator, endogenous pyrogen, serum amyloid A inducer, hepatocyte-stimulating factor, and interleukin-1 (1, 6). The last name tends now to be the most widely used (7). How these mediators interact with hepatocytes remains open to debate. According to the current concept, soluble mediators are released from the site of injury and transported by the blood to the liver where they interact with the hepatocytes (1). However, recent studies suggest that alternative pathways may exist. *In vitro,* peripheral blood monocytes (8) and hepatic sinusoidal macrophages (9–11) secrete factors which increase the hepatocyte synthesis of certain APR, suggesting that sinusoidal macrophages could be involved in the acute inflammatory reaction (12). The commitment of these cells *in vivo* may result from different mechanisms:

— the recruitment into the liver of peripheral blood monocytes that secrete a hepatocyte-stimulating factor;

— the multiplication of resident macrophages which synthesize mediators increasing the production of APR;

— the activation of resident macrophages without proliferation.

Thus, the quantitation of sinusoidal macrophages appeared to us as a first attempt to investigating the mechanisms of hepatocyte stimulation *in vivo.* This study was designed to determine whether quantitative changes could be detected in hepatic sinusoidal macrophages (HSM) during the initial stages of a turpentine-induced acute systemic inflammation in the rat and the rabbit.

Materials and Methods. *1. Experimental procedure.* An acute inflammatory reaction was induced by the subcutaneous injection of sterilized turpentine (Prolabo, Paris, France) in six male rabbits weighing 2 kg (0.3 ml/100

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g body wt) and in six male adult Sprague–Dawley rats weighing 250 to 280 g (0.5 ml/100 g body wt). This model was thoroughly investigated in previous experiments (13–17). It has been previously shown in our laboratory (13) that a synchronous increase in the concentrations of several acute phase reactants (i.e., fibrinogen, α-2-macroglobulin, haptoglobin, and α-1-acid glycoprotein) could be detected in liver homogenates by 16 hr after the injection of turpentine and reached a plateau by 24 hr. In the plasma, the maximum increase was observed by 40 hr (13). Three animals of each species were sacrificed according to a schedule corresponding to these kinetics, i.e., 16 and 24 hr after the injection. All animals were sacrificed at the same time of day.

Two types of controls were used. Three healthy animals of each species were sacrificed in order to determine the number of HSM in the normal state. Three other animals of each species were injected with saline and represented time 0 of the reaction. For each animal, three liver samples were taken from the main lobes.

2. Materials and methods: 2.1. Histological methods. Liver samples were fixed in Bouin's solution and embedded in paraffin. Five-micrometer thick sections were stained with hematoxylin and eosin.

2.2. Identification of sinusoidal macrophages. Liver sections were examined at a magnification of 1000× under oil immersion (Fig. 1). Sinusoidal macrophages were identified on morphological grounds, according to the following criteria (18): (i) cells attached to the sinusoidal wall and lying within the lumen of the sinusoid, (ii) stellate shape, (iii) large amounts of cytoplasm, with poorly defined limits and sometimes a foamy appearance, (iv) ovoid nucleus with dispersed chromatin.

The other sinusoidal cells, i.e., endothelial cells and fat-storing cells , were distinguished by their location and cytological characteristics (18, 19). Endothelial cells line the sinusoid and possess an elongated, dark nucleus with indistinct cytoplasm. Fat-storing cells are located

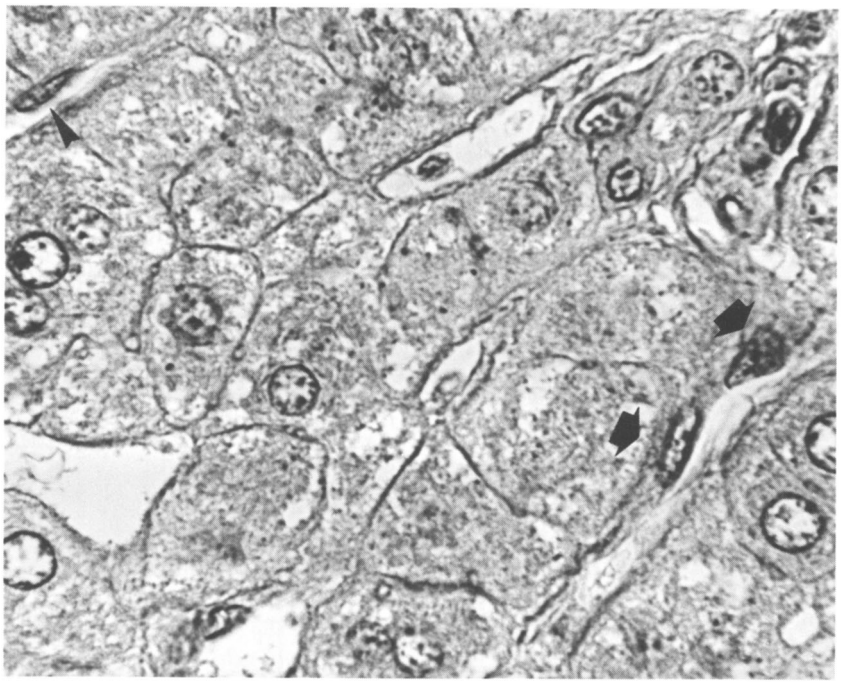

FIG. 1. Periportal area of the hepatic lobule in a control rabbit (Time 0 of the experiment). Hepatic sinusoidal macrophages (large arrows) and endothelial cells (arrowheads) exhibit distinctive morphological features. (Hematoxylin-eosin; magnification 1600).

at the angle between two hepatocytes. Cells located immediately close to the portal tracts were excluded from the count in order to avoid confusion with fibroblasts or portal inflammatory cells. The presence of mitotic cells and of phagocytic macrophages, characterized by large intracytoplasmic vacuoles containing ingested material, was noted.

2.3. Quantitation of sinusoidal macrophages. The counts were performed in fields measuring 0.082 mm^2. Eight periportal and eight perivenous fields were examined in each section. Periportal and perivenous fields were defined as the areas centered by a portal tract or by a centrolobular vein, respectively. The parenchymal surfaces were roughly comparable from one field to another. Each periportal or perivenous field was chosen in a different lobule. One section per block was evaluated, so that a total of 48 fields were examined for each animal.

The ratio between the periportal and the perivenous counts (PP/PV ratio) was calculated for each animal.

Reproducibility was assessed by comparison between the counts of the two observers: the concordance was 95%.

2.4. Statistical analysis. Results were analyzed with the nonparametric Mann–Whitney *U* test (20).

Results. *1. Morphological observations.* No differences in morphological features could be observed (Figs. 1 and 2). The number of phagocytic cells was the same at 0, 16, and 24 hr following the induction of inflammation. No mitotic cells were observed at any time.

2. Quantitation of HSM: 2.1. Rats (Fig. 3). In the three controls, the mean numbers of HSM per field were 4.9 ± 0.8, 4.6 ± 1.1, and 4.5 ± 1.2 in the periportal areas and 3.6 ± 0.9, 3.8 ± 0.8, and 4.1 ± 1.2 in the perivenous areas. PP/PV ratios were 1.2, 1.1, and 1.1, respectively.

At Time 0, the mean numbers of HSM per field were 5.1 ± 1.0, 4.0 ± 1.6, and 4.5 ± 1.4 in the periportal areas and 3.4 ± 1.5, 3.5 ± 1.1, and 3.5 ± 1.3 in the perivenous areas. PP/PV ratios were 1.5, 1.3, and 1.2, respectively.

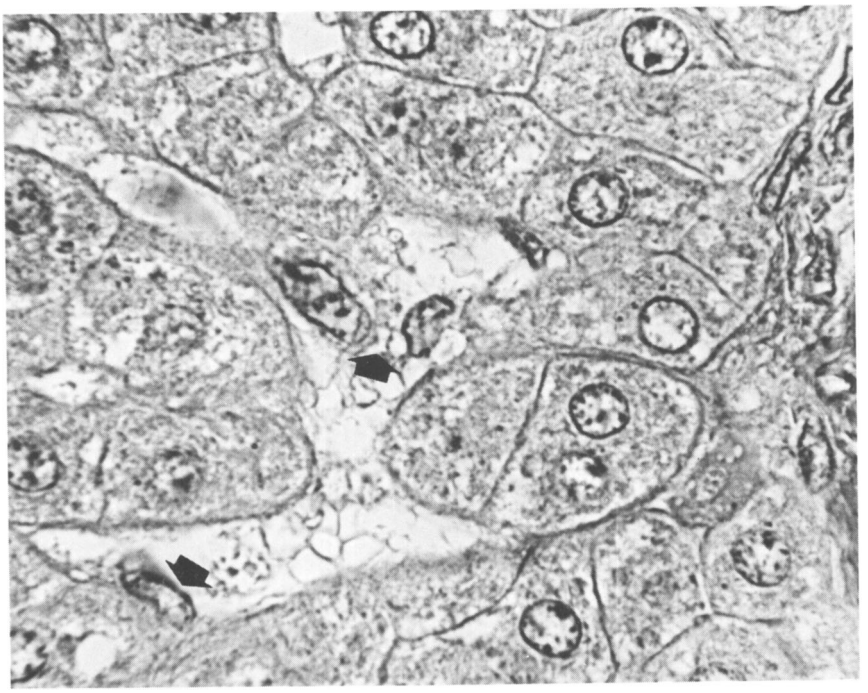

FIG. 2. Periportal area of the rabbit hepatic lobule, 16 hr after the induction of the inflammation. No variations in the morphologic characteristics of the hepatic sinusoidal macrophages (arrows) can be observed. (Hematoxylin-eosin; magnification 1600).

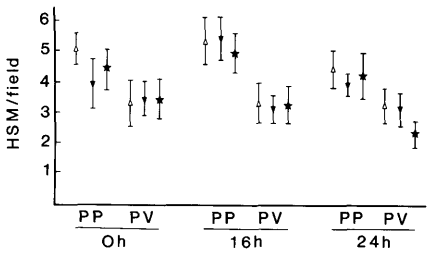

FIG. 3. Variations in the number of hepatic sinusoidal macrophages (HSM) per field in the periportal (PP) and the perivenous (PV) areas of the rat hepatic lobule, at the different times of a turpentine-induced inflammation. The number of HSM in the three controls (not figured) is not different from that observed at Time 0 of the reaction (see text). The three animals sacrificed at each time are represented by different symbols. No significant differences are found, either between the mean numbers of HSM per field, or between the PP/PV ratios.

At 16 hr, the mean numbers of HSM per field in the periportal areas were 5.4 ± 1.5, 5.5 ± 1.4, and 5.0 ± 1.2. In the perivenous areas, they were 3.4 ± 1.3, 3.2 ± 0.9, and 3.3 ± 1.2. PP/PV ratios were 1.6, 1.5, and 1.8, respectively.

At 24 hr, the mean numbers of HSM per field were 4.5 ± 1.2, 4.0 ± 0.7, and 4.3 ± 1.5 in the periportal areas. They were 3.4 ± 1.0, 3.2 ± 1.1, and 2.4 ± 0.9 in the perivenous ones. PP/PV ratios were 1.5, 1.3, and 1.4, respectively.

Statistical analysis did not show any significant difference between the mean numbers of HSM per field at 0, 16, and 24 hr. The comparison between the PP/PV ratios likewise showed no significant variation.

2.2. Rabbits (Fig. 4). In the three controls, the mean numbers of HSM per field were 5.1 ± 0.7, 4.4 ± 1.3, and 4.7 ± 1.0 in the periportal areas and 3.3 ± 1.1., 3.5 ± 0.9, and 3.0 ± 0.7 in the perivenous areas. PP/PV ratios were 1.4, 1.1, and 1.5, respectively.

At Time 0, the mean numbers of HSM per field were 4.7 ± 0.9, 4.8 ± 1.3, and 3.6 ± 1.1 in the periportal areas and 3.7 ± 0.9, 3.3 ± 1.1, and 2.6 ± 0.8 in the perivenous areas. PP/PV ratios were 1.2, 1.4, and 1.5, respectively.

At 16 hr, the mean numbers of HSM per field in the periportal areas were 5.1 ± 1.0, 4.7 ± 1.2, and 4.8 ± 1.3. In the perivenous areas, they were 4.1 ± 1.0, 3.8 ± 1.1, and 3.9 ± 1.2. PP/PV ratios were 1.2, 1.2, and 1.2, respectively.

At 24 hr, the mean numbers of HSM per field were 4.5 ± 1.2, 4.3 ± 1.5, and 4.8 ± 1.1 in the periportal areas. They were 4.3 ± 1.5, 3.7 ± 1.1, and 4.2 ± 1.1 in the perivenous areas. PP/PV ratios were 1.1, 1.2, and 1.1, respectively.

No significant differences were observed among the mean numbers of HSM per field at 0, 16, and 24 hr. The PP/PV ratios also remained within the same range.

Discussion. Several agents are known to induce an increase in the population of HSM. Some of them, such as zymosan, glucan, and stilboestrol, are nonspecific stimulators of the monocyte–macrophage lineage (21, 22). Others, such as *Corynebacterium parvum* and endotoxin, reproduce the effects of enterically derived antigens (23). The increase of HSM population induced by these agents was shown to result from two associated mechanisms, the influx into the liver of newly recruited macrophages and the multiplication of resident HSM (21, 22). However, little is known about the quantitative variations of HSM during the acute systemic inflammation. In order to determine whether the acute phase is accompanied by an influx into the liver of newly recruited macrophages or by the proliferation of resident HSM, we quantify the HSM in a model of acute systemic inflammation.

Our results do not support the hypothesis of an influx of newly recruited macrophages into the liver. We found no significant difference between the numbers of HSM at 0, 16,

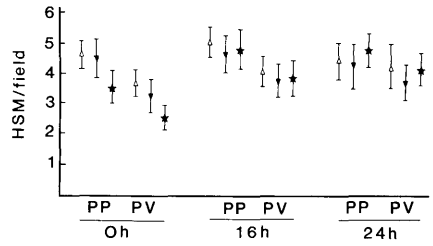

FIG. 4. Variations in the number of hepatic sinusoidal macrophages (HSM) per field in the periportal (PP) and the perivenous (PV) areas of the rabbit hepatic lobule, at the different times of a turpentine-induced inflammation. The number of HSM in the three controls (not figured) is comparable to that observed at Time 0 of the reaction (see text). The three animals sacrificed at each time are represented by different symbols. There are no significant variations in the number of HSM per field or in the PP/PV ratios.

and 24 hr during a turpentine-induced inflammation in two animal species. The period between 16 and 24 hr is the critical phase of the hepatic response to inflammation. As shown by previous investigations, the hepatic concentrations of the major APR rise synchronously between 16 and 24 hr and reach a plateau by 24 hr (13). That the HSM population was found constant at 16 and 24 hr indicates that no massive recruitment of monocytes occurred either before or during this phase of the hepatic response to inflammation.

The present results allow us to eliminate the hypothesis that resident cells proliferate during the initial stages of the inflammatory process. No increase in the number of mitotic cells was observed under the experimental conditions used here. HSM differ from the other resident macrophages in that they possess a capacity of self-renewal after stimulation (24). However, since the mean doubling time of these cells is about 50 hr (24), changes in hepatocyte protein synthesis take place much earlier than the first detectable changes in HSM proliferating activity (25).

It is known that in the normal state all hepatocytes are able to synthesize and secrete APR: no real differences between cells from periportal and perivenous areas have been convincingly demonstrated (3). However, certain investigations have suggested that hepatocytes become progressively committed to APR synthesis during the acute inflammatory reaction (13, 15). In the early period (10–16 hr), APR-synthesizing hepatocytes seem to be located mainly in the periportal areas, while later (24 hr) they could be detected in nearly the entire hepatic lobule. We found no parallel variation in the distribution of the HSM during the acute inflammatory reaction. The ratios of HSM counts in the periportal and perivenous areas remain within the same range at 0, 16, and 24 hr. They are comparable to the normal ratio of 4:3:2 measured by Sleyster and Knook (26) among the periportal, midzonal, and perivenous zones of the rat hepatic acinus. In this study, we did not individualize a midzonal zone as the main differences in distribution and function of HSM were observed by these authors between the periportal and the perivenous zones.

The absence of detectable quantitative changes in the HSM population during the acute inflammatory reaction does not necessarily mean that these cells do not undergo qualitative changes during this period. In this way, the recruitment of a small number of stimulated blood monocytes cannot be definitively excluded. Their identification within the resident population would require the ultrastructural localization of peroxidase, which is considered to be different from that observed in resident HSM (27).

An activation of resident cells, without multiplication, could also be postulated. Various definitions have been proposed for "activated" macrophages. Metabolic changes are the most characteristic features of these cells. Activated macrophages show increased oxygen consumption, calcium uptake, and hexose monophosphate shunt activity. They undergo a respiratory burst which results in the production of oxygen species (23, 27, 28). Such biochemical changes are difficult to identify by morphological methods. Variations in certain enzyme activities are associated with the metabolic burst. Increasing *N*-acetyl-glucosaminidase, lysozyme, and in certain studies, acid phosphatase and β-galactosidase activities have been detected in stimulated HSM maintained in primary cultures (29–31).

Histochemical methods are available for the detection of these enzymes in tissue sections. However, some of these techniques lack of sensitivity (32) and specificity (33). In any case, the quantitation of histochemical results is difficult. Moreover, it is not known whether activated macrophages *in situ* undergo the same variations in enzyme activities than cells maintained in culture. Indeed, culture conditions can promote enzymatic variations despite the absence of exogenous stimulation (34, 35). Finally, surface membrane modifications have been detected recently in activated macrophages using monoclonal antibodies (36–39). Certain of these antibodies would be able to distinguish inflammatory macrophages from resting ones (37, 38). However, they are not yet available.

In conclusion, the increased hepatocyte synthesis of APR during the acute inflammatory reaction is not accompanied either by an increase in the HSM population or by variations in the distribution of HSM inside the lobule. These results suggest that the mechanism of hepatocyte stimulation during the initial stages of the inflammatory process does not involve hepatic recruitment of monocytes.

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