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Letter to the Editor

The liver sinusoidal endothelium reappears after being eclipsed by the Kupffer cell: a 20th century biological delusion corrected

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The midpart of the 20th century saw a perplexing period of scientific progress in innate immunity. The interpretation I present was suggested in broad outline by the prescient reviews of Bard Smedsrod and others [1]. I have supplied additional supporting evidence [2, 3].

Recent work from our laboratory has rekindled a new way of understanding how small particles, such as viruses and small immune complexes, are removed from blood (clearance); namely, they are eliminated rapidly and extensively by the endothelium of liver sinusoids (LSEC) and to a much lesser extent, by KC. Whereas exploration of this process began in the early years of the 20th century, pursuit was soon abandoned, and today, next to nothing is known.

Why study of the clearance process was delayed for 50 years defies a simple explanation. Three reasons are possible. First, virologists are focused intently on infection, and as clearance does not involve infection, clearance is thus far outside their purview. Second, clearance is so fast and complete that it would seem futile, at first blush, to hope to hasten it for therapeutic purposes. Third, the delay would appear to derive, in part, from a longstanding and pervasive misconception about the nature and function of the scavenger cells of the hepatic sinusoid, the cells largely responsible for virus elimination from blood. KC have long been thought responsible, whereas now it appears likely that LSEC are the main scavengers. All that we have learned about KC uptake of virus may be irrelevant.

The details of the delay in discovery are these: early 20th century biologists, studying the uptake of intravenously infused colloidal stains, noted the remarkable scavenging properties of the liver sinusoidal cells. These cells were a major feature of the RES, a term coined by Aschoff in his 1924 review [4]. The RES is a collection of diverse cells in several organs responsible for clearing the circulation of all waste macromolecules and particles (not only viruses but small immune complexes). In the liver, the RES consists of both types of scavenger cells of the liver sinusoid, the KC and the LSEC. The liver sinusoids, it should be noted, constitute a huge blood-vessel network that serves as the liver conduit for the entire volume of hepatic portal blood plus an additional 20% of systemic blood—fully one-third of the cardiac output. These liver sinusoids are lined by a distinctive endothelium (LSEC) that is decorated on the luminal side by less-numerous KC and is separated from hepatocytes on its basal side by the space of Disse [5].

Gradually, during the mid-20th century, one of these sinusoidal cells—the LSEC—was forgotten, lost from scientific consciousness. Its place was taken by the other sinusoidal scavenger, the KC. How this switch happened is curious and informative: it would appear that the world became single-mindedly enamored with the phagocyte. The KC, known to be vigorously phagocytic, was subsumed into the newly coined MPS. Defined with influential authority by van Furth and prominent colleagues in 1972 [6], the MPS included tissue macrophages, monocytes, and their precursors. However, the LSEC were excluded from the MPS by definition because they failed to phagocytose in the modern sense of the term; they were only pinocytic.

[We define pinocytosis as the uptake of small (<0.5 μm) particles into vesicles, independently of actin filaments, and phagocytosis as the uptake of larger particles (>0.5 μm) by a process involving actin polymerization. Both can be receptor mediated. Both are types of endocytosis, a more general term meaning uptake into a cell. Aschoff and Metchnikov [4] used the term "phagocytosis" to mean endocytosis, unable to distinguish between pinocytosis and phagocytosis. Only later was the term "pinocytosis" introduced, by Lewis in 1934, and the distinction between pinocytosis and phagocytosis was apparent.]

Our knowledge of the MPS burgeoned as part of the revolution in biology of the last 50 years, whereas work on LSEC led an independent but fragile life, pursued by the cognoscenti but largely ignored by those interested in the MPS and by the mainstream of immunology and virology. For example, an influential 1964 review on viral pathogenesis credits all liver clearance to the KC and makes no mention of LSEC [7]. Furthermore, the popular textbook *Viral Pathogenesis*, edited in 1997 by N. Nathanson [8], misrepresents the actual anatomy of the liver sinusoid in a cartoon on page 22 by omitting LSEC altogether, illustrating the KC as the cell lining the sinusoid.

Abbreviations: KC = Kupffer cell(s), LSEC = liver sinusoidal endothelial cell(s), MPS = mononuclear phagocyte system, RES = reticuloendothelial system

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A more astonishing, putative manifestation of the apparent cultural shift in view was the 1980s' change in names of the *Journal of the Reticuloendothelial Society* to the *Journal of Leukocyte Biology*. We biologists appear to have suffered a widespread, conceptual misunderstanding that some might consider a delusion [9].

Thus, we were gratified recently to learn of the restoration of the original definition of the liver RES as the sum of the MPS and the LSEC [1]. The elegant experiment [10] that resurrected the LSEC from obscurity was the examination with modern imaging methods, including electron microscopy, of livers from rats infused with lithium carmine, an RES stain used by Aschoff and colleagues a century earlier. It was learned that LSEC were vigorous scavengers, rivaling and even surpassing KC, stuffed so full that the dye protruded out into the lumens of the sinusoids, causing the LSEC to resemble KC. Yet, the LSEC showed keen distinctions from KC that provided new insight into their nature. The chief distinction appears to be that LSEC, unlike KC, are not phagocytic, but they are vigorously pinocytic, taking up small particles, less than ~0.5 μm, which would include all viruses as potential pinocytic targets (a point virtually everyone had ignored earlier). They contain abundant coated vesicles and display a variety of endocytic receptors, including mannose, collagen, hyaluronan, scavenger, L-SIGN (liver/lymph node-specific intercellular adhesion molecule-3-grabbing nonintegrin), and FcRs (FcγRIIb), but not complement receptors (see review, ref. [1]). Highly attenuated, perforated by clusters of patent fenestrae, full of lysosomes, and lacking a basement membrane, they are estimated to be more voluminous and numerous than KC and, magnified by liver size, to constitute a deceptively large adsorptive surface area. Furthermore, they present antigen to produce T cell tolerance.

According to a recent phylogenetic study, scavenger endothelial cells (such as LSEC) are expressed throughout the vertebrate kingdom and in insects, which to us suggests that this group of cells might profitably be included as an additional "module" of the innate immune system as defined recently by Medzhitov [11]. For sake of clarification, we add that viruses, as a rule, are small enough to be cleared by the process of pinocytosis by LSEC and KC, but should virions be aggregated, by whatever means, they may be too large for pinocytic uptake and would then qualify for phagocytic uptake by KC but not LSEC.

An exploration of LSEC-mediated clearance of small particles has begun. Of late, LSEC-mediated clearance has been shown for adenovirus [2] and polyoma viruses and ~10 other virus reports, which can be gleaned from the old literature. This progress promises an updating of what we know about the hematogenous spread of virus. Circulating small immune complexes, <200 nm in diameter, are cleared from blood almost exclusively (90%) by the LSEC rather than KC, suggesting a new look at nascent antibody-mediated autoimmune disease [3]. Very likely, nanoparticles of many sorts, known for years to be cleared from the blood immediately, are likely taken up by LSEC.

The endothelium of the liver sinusoid is becoming known as a potent outpost of the innate immune system. The mechanism of elimination from blood of virus and other small particles is due for a thorough evaluation of its component cellular and molecular details, an evaluation initiated 100 years ago and then promptly abandoned. Such an understanding will bring new strategies for modulating clearance rates, which in turn will likely lead to novel therapeutic approaches.

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KEY WORDS:
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