A 20th Century Biological Delusion.

Clark L. Anderson and Latha Ganesan

November 3, 2014.

I have extracted the following short perspective from my grant applications and will archive it on my website, here, until at some time in the near future I am able to include it in a review of this area. I believe it is an important historical interpretation that explains a lengthy period of puzzling literature. It was suggested to me in broad outline by the reviews of Bard Smedsrod; I have supplied additional supporting evidence.

Recent work from my laboratory has supported a new way of understanding how small particles such as viruses are cleared from the blood; namely, that they are eliminated very quickly and extensively by the endothelium of the liver sinusoids (LSEC), and to a much lesser extent by Kupffer cells (KC). While exploration of this process began in the early years of the 20th century, pursuit was quickly abandoned and today almost nothing is known.

Why the study of the clearance process has been delayed for 50 years defies a simple explanation. Three reasons are possible. First, virologists seem focused intently on infection, and clearance does not involve infection and thus is 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[1]. KC have been thought responsible, in short, whereas it appears that LSEC are likely the main scavenger. All that we have learned about KC uptake of virus may be irrelevant.

The story of the delay is this: Early 20th century biologists, studying the uptake of intravenously infused colloidal stains, were aware of the remarkable scavenging properties of the liver sinusoidal cells. These cells were a major feature of the reticuloendothelial system (RES), a term coined by Aschoff in his 1924 review[2]. 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, both the KC and the LSEC. The liver sinusoids, it should be noted, constitute a huge blood vessel networkthat 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[3-5].

Gradually, during the mid-20th century, one of these sinusoidal cells, the LSEC, was forgotten, was 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 *M*ononuclear *P*hagocyte *S*ystem (MPS). Defined with influential authority by Van Furth and prominent colleagues in 1969[6], the MPS included tissue macrophages, monocytes, and their precursors. But the LSEC were

excluded from the MPS by definition because they failed to phagocytose[a] in the modern sense of the term; they were only pinocytic. Our knowledge of the MPS burgeoned as a result of the biology revolution of the last 50 years, while 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[9]. Further, the popular textbook on *Viral Pathogenesis* edited in 1997 by N. Nathanson misrepresents the actual anatomy of the liver sinusoid in a cartoon on p22 by omitting LSEC altogether, illustrating the KC as the sinusoidal lining cell[10]. A more astonishing apparent manifestation of the cultural shift in view was the 1980s change in names of the Society and Journal of the RES to the Society and Journal of Leukocyte Biology[11]. We appear to have suffered a widespread conceptual misunderstanding that some might call a delusion.

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[12,13]. The elegant experiment that resurrected the LSEC from obscurity was the infusion of rats with lithium carmine, an RES stain used by Aschoff and colleagues a century earlier, followed by the examination of the organs with modern imaging methods including electron microscopy[1]. It was revealed that LSEC were vigorous scavengers rivaling and even surpassing KC, stuffed so full of dye as to protrude out into the lumens of the sinusoids, thus resembling 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 micron (um), which would include all viruses as potential pinocytic targets (a point virtually everyone had earlier ignored). They contain abundant coated vesicles and display a variety of endocytic receptors, including mannose, collagen, hyaluronan, scavenger, L-SIGN, and Fc receptors (Fc□ RIIb); but not complement receptors (review)[13]. Highly attenuated, perforated by clusters of patent fenestrae, full of lysosomes, and without 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. Further, they present antigen to produce T cell tolerance (reviewed)[14].

According to a recent phylogenetic study, scavenger endothelial cells (like LSEC) are expressed throughout the vertebrate kingdom and in insects[15], 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[16]. 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.

The field of virus elimination needs a thorough evaluation of its component cellular and molecular details, an evaluation that was initiated 50 years ago and then promptly abandoned. Such an understanding will bring new strategies for modulating clearance rates, which in turn will lead to novel therapeutic approaches.

---

[a]We define pinocytosis as the uptake of small (< 0.5 um) particles into vesicles independently of actin filaments, and phagocytosis as the uptake of larger particles (> 0.5 um) by a process involving actin polymerizatioin. Both can be receptor mediated. Both are types of endocytosis, a more general term meaning uptake into a cell[7].Aschoff and Metchnikov used the term 'phagocytosis' to mean endocytosis, unable to distinguish between pinocytosis and phagocytosis[2]. Only later was the term 'pinocytosis' introduced, by Lewis in 1934[8], and the distinction between pinocytosis and phagocytosis was apparent.

Reference List

1 Wake,K., Kawai,Y., & Smedsrod,B. Re-evaluation of the reticulo-endothelial system. **106**, 261-269 (2001).
2 Aschoff,L. Reticuloendothelial System (Janeway Lecture, New York) in *Lectures on Pathology (delivered in the United States, 1924)* 1-33 (Paul B. Hoeber, Inc., New York, 1924).
3 Wisse,E. An ultrastructural characterization of the endothelial cell in the rat liver sinusoid under normal and various experimental conditions, as a contribution to the distinction between endothelial and Kupffer cells. **38**, 528-562 (1972).
4 Hubbard,A.L. & Stukenbrok,H. An electron microscope autoradiographic study of the carbohydrate recognition systems in rat liver. II. Intracellular fates of the 125I-ligands. *J. Cell Biol.***83**, 65-81 (1979).
5 Hubbard,A.L., Wilson,G., Ashwell,G., & Stukenbrok,H. An electron microscope autoradiographic study of the carbohydrate recognition systems in rat liver. I. Distribution of 125I-ligands among the liver cell types. *J. Cell Biol.***83**, 47-64 (1979).
6 Van Furth,R. *et al.* The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. **46**, 845-852 (1972).
7 Ravetch,J.V. & Anderson,C.L. Antibody-mediated endocytosis: FcγR family: proteins, transcripts, and genes in *Fc receptors and the action of antibodies* (ed. Metzger,H.) 211-238 (Am.Soc.Microbiol., Washington,D.C., 1990).
8 Lewis,W.H.R. Pinocytosis. **49**, 17-26 (1931).
9 Mims,C.A. Aspects of the pathogenesis of virus diseases. **28**, 30-71 (1964).
10 Nathanson,N. & Tyler,K.L. Entry, dissemination, shedding, and transmission of viruses in *Viral Pathogenesis* (ed. Nathanson,N.) 13-32 (Lippincott Williams and Wilkins, 1997).
11 Stewart,C.C. From the Editor. **35**, front matter (1984).
12 Smedsrod,B., Pertoft,H., Gustafson,S., & Laurent,T.C. Scavenger functions of the liver endothelial cell. *Biochem. J.***266**, 313-327 (1990).
13 Elvevold,K., Smedsrod,B., & Martinez,I. The liver sinusoidal endothelial cell: a cell type of controversial and confusing identity. **294**, G391-G400 (2008).
14 Kern,M., Popov,A., Kurts,C., Schultze,J.L., & Knolle,P.A. Taking off the brakes: T cell immunity in the liver. **31**, 311-317 (2010).
15 Seternes,T., Sorensen,K., & Smedsrod,B. Scavenger endothelial cells of vertebrates: a non-peripheral leukocyte system for high capacity elimination of waste macromolecules. **99**, 7594-7597 (2002).
16 Medzhitov,R. Recognition of microorganisms and activation of the immune response. *Nature***449**, 819-826 (2007).