Generation and Use of a Mouse Kupffer Cell Line

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Summary
Kupffer cells are the most abundant population of tissue macrophages, involved in the pathogenesis of various liver diseases. We generated a murine Kupffer cell line from H2Kb-tsA58 transgenic mice, which has proved useful for the study of drug delivery, of phagocytosis of pathogens and apoptotic cells and of the role of KC in transplantation.

Keywords: liver macrophages, Kupffer cell line, mouse

Background Information

What are Kupffer cells?

Kupffer cells account for 80 to 90% of resident macrophages in the body, they constitute about 15% of the liver cells. Kupffer cells are located along the sinusoid and exhibit different phenotypes and functional capacity in the periportal, intermediate and central area of the liver lobule (Fig. 1A). They have a life span of approximately 14 months and only a small fraction, amounting to 3%, is responsible for cell population renewal in vivo.

The principal role of Kupffer cells is phagocytosis and mediation of the innate immune response in the liver. Kupffer cells are able to remove from the blood any particulate or soluble gut-derived stimulus (Fig. 1B); upon uptake they release reactive oxygen species, nitric oxide, cytokines and lipid mediators. The best known stimulus of Kupffer cells is the gram-negative cell membrane constituent, Lipopolysaccharide (LPS) or endotoxin, which is a normal component of portal venous blood. Depending on the timing, frequency and intensity of endotoxin stimulation, sensitisation or tolerance to endotoxin results. Excessive stimulation of Kupffer cells leads to liver damage via release of cytokines and toxic oxygen products.

Impact of Kupffer cells in disease

Kupffer cells play an important role in a number of diseases:
Sepsis: During early sepsis, Kupffer cells – together with granulocytes, which accumulate in liver sinusoids – participate in bactericidal activity and release reactive oxygen and proteolytic products, which are a cause of liver injury.
Alcohol induced injury: Due to alcohol induced, impaired gastrointestinal epithelial barrier function, Kupffer cells become exposed to increased levels of endotoxin, which cause a sustained production of inflammatory mediators. Consequently, Kupffer cell sensitivity to LPS is enhanced and contributes to chronic alcohol hepatitis.
Hepatectomy: After partial hepatectomy for liver tumor resection, liver regenerates and all cells including Kupffer cells proliferate, these have a controversial effect on the growth of other liver cells.
Liver transplantation: Kupffer cells interact with circulating T cells in the transplanted liver. Recent experiments in allograft models have shown, that Kupffer cells induce tolerance by suppressing T cell proliferation and inducing T cell apoptosis. Also, Kupffer cells isolated from an accepted allograft, prolong liver survival in a rejection model.
Ischemia/reperfusion injury during liver surgery or transplantation: During liver surgery, perfusion may be interrupted by periods of ischemia; this causes irreparable damage to which Kupffer cells participate. Pre-exposure of the liver to transient ischemia increases the tolerance to reperfusion. This process is mainly mediated by Kupffer cells, which produce less proinflammatory cytokines and attract less granulocytes after ischemic preconditioning.

Together this demonstrates that it is highly relevant to know more concerning the function and role of this particular liver cell population.

Why immortalised Kupffer cells?

Investigations concerning Kupffer cells are hampered, because in humans, Kupffer cells are only accessible for immunohisto-

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chemical analysis, from biopsies or autopsies. From rats and mice they are difficult to isolate and after purification only approx. 5 million cells can be obtained from one mouse. Furthermore, these Kupffer cells are not homogeneous, form syncytia, rarely proliferate and survive for not more than 10 days. These reasons have made it mandatory to search for a way to immortalise Kupffer cells while preserving their original function.

How to generate a Kupffer cell line

Kupffer cells were isolated from H-2K^b-tsA58 transgenic mice, which stably express a thermolabile mutant of the Simian virus 40 (SV40) large tumor antigen under the control of a histocompatibility gene promoter (H-2K^b). Cells isolated from this mouse grow continuously at the permissive temperature of 33°C, at which the mutant tsA58 is active, but don't grow, or grow less under the normal culture temperature of 37°C. Growth is initiated by the incubation of cells with interferon-γ, which activates histocompatibility genes.

In the present project, we harvested Kupffer cells by collagenase perfusion of the liver, gradient centrifugation and subsequent counterflow centrifugation. Four lines were generated by culture at 33°C, in a medium containing interferon-γ and conditioned media from a hepatocyte and an endothelial cell line. In the absence of these paracrine growth factors, we observed a gradual loss of phenotype and secretory function.

One out of 4 clones (KC13-2) obtained by limiting dilution from the line, grew stably at 33ºC without interferon-γ and also – although slower – at 37°C, which indicates an interferon-γ and temperature-independent regulation of SV40T Ag. Specific Kupffer cell characteristics of the KC13-2 clone were confirmed by comparing phenotypes and functions between peritoneal macrophages, primary isolated Kupffer cells and our clone. We had to use 44 mice to establish and characterise the clonal line. In comparison: When working with primary Kupffer cells, a single experiment requires the livers of 10 mice and the variability between experiments is large.

What are these cells?

The generated cell line has been growing in culture in a stable manner for more than 6 years. The cells of the clone, in contrast to the line and primary cell, were uniform, survived detachment and could therefore be analysed by flow cytometry. The KC13-2 clone, like the primary Kupffer cells, constitutively expressed a number of specific functions and structures. These include: a) the classical macrophage enzymes non-specific esterase (Fig. 2A) and peroxidase; b) two macrophage-specific antigens of unknown function, MOMA-2 (Fig. 2B) and BM8 (identical to the antigen known as F4/80); c) the pattern recognition receptors, which are activated by pathogen-associated conserved molecules, including scavenger receptor A, CD14 and Toll-Like-Receptor 4/MD-2 (TLR4/MD-2) (Fig.3); d) the antigen presenting molecules MHC class I and II, CD40, CD80 and CD1d; e) Kupffer cells endocytosed Dextran-FITC, which is another characteristic of immature antigen presenting, dendritic cells; f) the lack

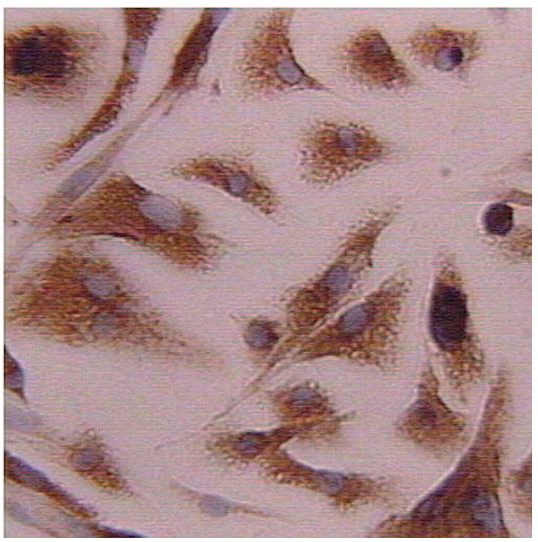

Fig. 1A: Primary Kupffer cell cultured *in vitro*: Stain with macrophage-specific antibody.
Cell size is not homogenous, because Kupffer cells from the periportal area are larger than those from intermediate or central-lobular area.

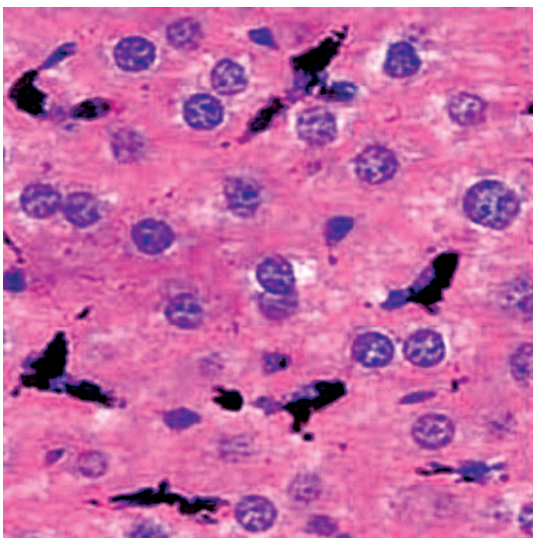

Fig. 1B: Histology of Liver Sinusoid:
Kupffer cells are stained in black after ink uptake, they are interposed between hepatocytes.

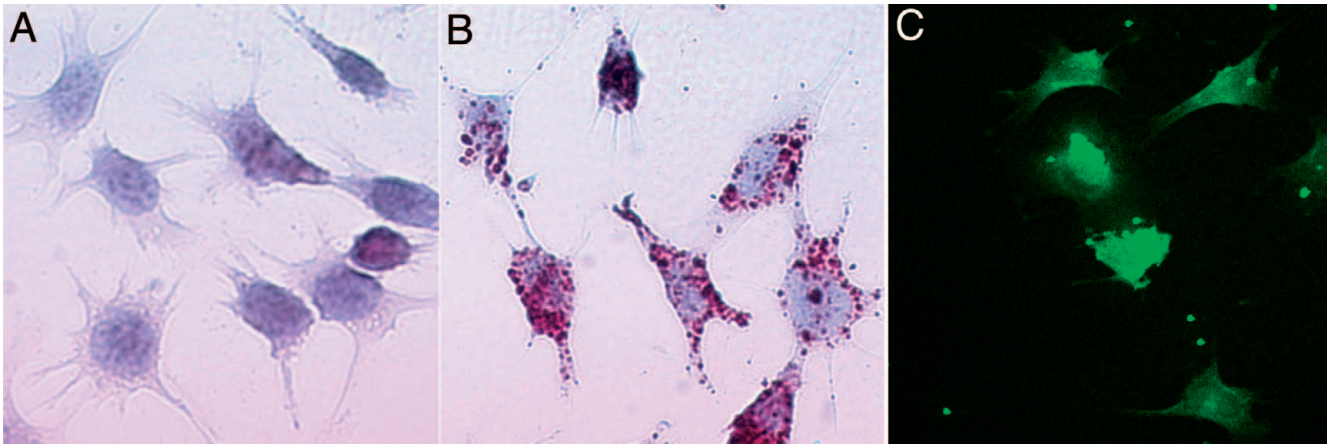

Fig. 2A: Enzymatic activity (esterase) of Kupffer cell line (KC 13-2).

Fig. 2B: Staining of Kupffer cell line KC 13-2 with macrophage specific antibody MOMA-2.

Fig. 2C: Phagocytosis of fluorescent *E.coli* by the Kupffer cell line KC 13-2 1h at 37°C.

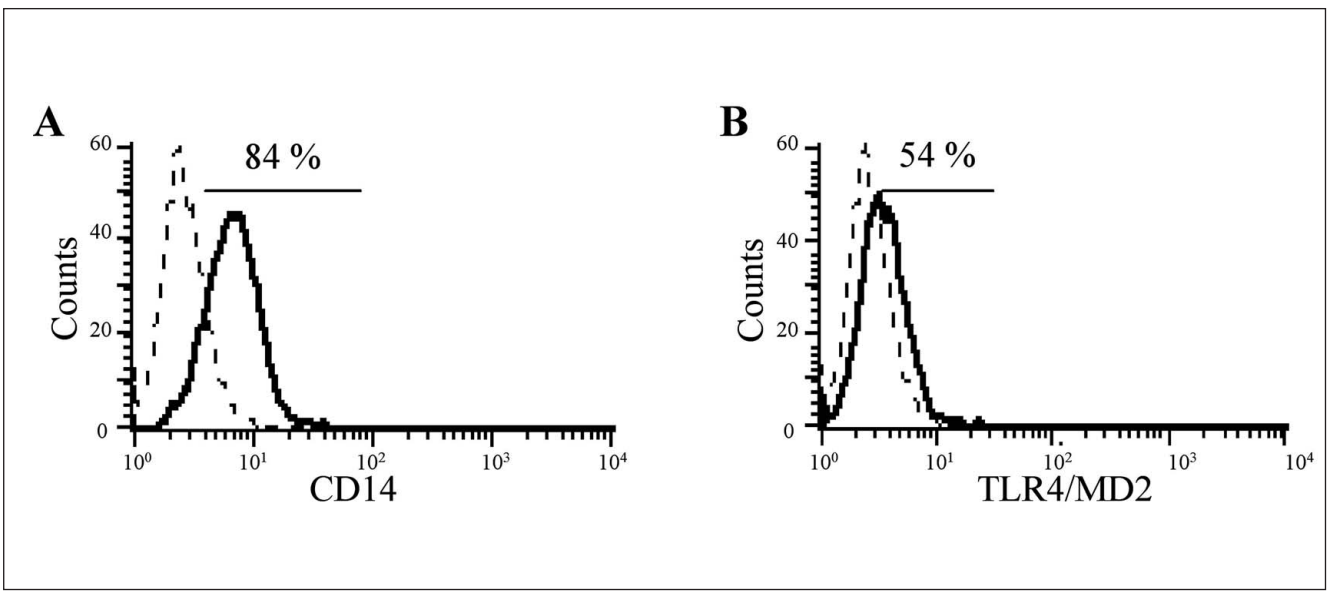

Fig. 3: Pattern receptor expression of CD14 (left) and TLR4 (right) in Kupffer cell line KC 13-2, dotted line: isotype control antibody staining.

of the phagosomal coat protein TACO, which is in all macrophages, except Kupffer cells; g) exhibition of CD14- and TLR4/MD2-independent, plasma-dependent lipopolysaccharide (LPS) binding. h) *E. coli* (Fig. 2C) and *S. pneumoniae* phagocytosis and LPS- and IFN-γ-induced NO production, but no TNF-α, IL-6 or IL-10 release (Dory et al., 2003).

In summary, the large size, surface marker expression and capacity to clear gram-negative and -positive bacteria, but absence of cytokine release, indicates that the clone was derived from the periportal large Kupffer cell subpopulation.

A valuable tool for mechanistic investigations

For the first time it has been possible to generate a stable, clonal Kupffer cell line representing a subpopulation within the Kupffer cells of rodent livers.

The applications for the use of the Kupffer cell line in disease models are multiple and scientists from all over the word asked for the line to study e.g.:

Liver disease after parasite infection: signalling in Kupffer cells after stimulation with bacteria or bacterial products is analysed

(Peng et al., 2006). Furthermore, parasite entry into Kupffer cells and the immunological response after uptake of apoptotic cells is followed.

Liver transplantation: The set of antigen presenting molecules in the clone allows the study of the consequences of antigen presentation to MHC- and CD1-restricted T cells. Furthermore it allows the investigation *in vitro* of xenogeneic interactions e.g. between murine Kupffer cells and human erythrocytes and consequences of antigen presentation, such as T cell apoptosis e.g. in liver transplantation.

Liver tumors: The role of Kupffer cell line in liver tumor formation is studied.

Drug delivery: Firms interested into drug delivery have started using the line for uptake studies.

The clone allows molecular studies of the antiinfective and immune functions of Kupffer cells. It will reduce and replace studies with primary Kupffer cells obtained from mice.

However, control of *in vitro* derived knowledge has to be proven in selected cases, in an intact animal or directly in specific clinical applications.

References

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