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Microchimerism in liver allografts: the swing of the pendulum

High frequency of chimerism in transplanted livers. Ng IO-L, Shek K-L, Lee JM-F, Fong DY-T, Lo C-M, Fan S-T.

Recent studies have shown that primitive stem cells can mobilize and differentiate into hepatocytes. We investigated the time and extent in which cells of recipient origins could differentiate into hepatocytes and other cells in human liver allografts. Microsatellite analysis, which can assess quantitatively the proportions of recipient and donor DNA, was performed in post-transplantation liver biopsy specimens from 17 patients at various times. Combined fluorescent in situ hybridization (FISH) for Y chromosome and immunofluorescence for different cell types was also performed in 10 of these cases with sex mismatch. Organ chimerism in the transplanted livers was found to be of variable extent, and the recipients' DNA in the post-transplantation liver biopsy specimens (excluding portal tracts) amounted up to 50%. The recipient DNA in the post-transplantation liver biopsy specimens increased after liver transplantation by as early as 1 week, peaked at around 30–40 weeks, and could be shown 63 weeks after transplantation. Most (64–75%) of the recipient-derived cells showed macrophage/Kupffer cell differentiation. Only up to 1.6% of the recipient-derived cells in the liver grafts showed hepatocytic differentiation in the liver grafts and made up 0.62% of all hepatocytes of both donor and recipient origins. These livers had mild or minimal injury histologically. In conclusion, our results show that most of the recipient-derived cells in the liver allografts were macrophages/Kupffer cells and only a small proportion of hepatocytes was recipient-derived. However, with regard to recipient-derived hepatocytes, our data cannot distinguish between transdifferentiation and cell fusion.

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In the field of liver transplantation, peripheral and intragraft microchimerism resulting from a two-way traffic of lymphocytes, macrophages and Kupffer cells between the liver graft and the host has been described since the early 1990s and considered to be responsible at least in part for the relative immunological tolerance of liver allografts [1].

From the first studies performed in the 1980s on intragraft microchimerism, it has been considered for decades that only Kupffer cells of recipient origin could be found within the transplant whereas endothelial cells, biliary cells and hepatocytes remained of donor origin [2]. More recently, a great renewal of interest for intragraft chimerism rose from the concept of plasticity of stem cells. Evidence was growing from animal models that bone marrow-derived cells can differentiate into hepatocytes [3] with functional role [4]. The issue of chimerism for epithelial cells within human liver grafts was then revisited [5–7] using more sensible and more precise methods such as fluorescent in situ hybridization (FISH) on interphase nuclei, markers for genetic polymorphism and tissue microdissection. These studies demonstrated for the first time that both hepatocytes and biliary epithelial cells within human liver allografts could originate from recipient marrow-derived cells. Parallely, it was shown that in livers of bone marrow recipients hepatocytes could originate from donor bone marrow cells [6]. Some discrepancies could be noted in the quantitative results. The level of hepatocyte chimerism in liver grafts was quite different from one study to another: 0.5–2% for Alison et al. [5] compared with 16–40% for Theise et al. [6]. These discrepancies could be explained at least in part by the degree of graft lesions for highest percentages of hepatocyte chimerism were observed in cases of severe recurrent hepatitis [6,7]. Nevertheless the difficulty of interpreting FISH positive signals in liver sections due to the abundant autofluorescent bilirubin and porphyrin metabolites has to be mentioned. Further studies on liver allografts [8,9] including the publication under consideration [10] reported a very low level of hepatocyte

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chimerism up to 1% or even nul. As noticed in the discussion by Ng et al. [10], recent studies from animal models confirmed that hepatocyte replacement by bone marrow-derived cells after bone marrow engraftment is a rare and slow event needing a selective pressure [11,12]. A lot of additional work will be then necessary to hope to reach a therapeutic level in the management of liver disease by cell therapy with bone marrow stem cells.

Besides the quantitative aspect, another point still in discussion is the mechanism by which hepatocytes derive from bone marrow cells. Precise caryotypic and molecular characterization of chimeric hepatocytes in the animal model of type 1 tyrosinemia [13,14] pointed out a mechanism of fusion between donor and recipient cells. The donor cell responsible for fusion would be a differentiated progeny of bone marrow stem cells initially transplanted in the donor, for example macrophage/Kupffer cell. Although Ng et al. [10] demonstrated that most Kupffer cells are recipient-derived cells, they failed as all the other studies performed in human liver allografts to find out any argument such as multinucleation or Y chromosome polysomy in favour of a fusion mechanism. Similarly, it was recently shown that human cord blood cells can differentiate into hepatocytes in the liver of NOD-SCID immunodeficient mice with no evidence of fusion [15]. Both mechanisms, i.e. true transdifferentiation and fusion are likely to be involved in these phenomena of cell plasticity.

During the last 4 years, all the efforts were concentrated upon the search for hepatocyte and cholangiocyte chimerism in human liver grafts. Now in a more general view of 'tissue biology' [16], interest is coming back towards non-epithelial cells as Kupffer and endothelial cells. In this work, Ng et al. [10] show that liver graft chimerism is constant even when avoiding lymphocytic infiltrates within portal tracts by microdissection procedure and is mainly due to recipient-derived cells demonstrating macrophage/Kupffer cell differentiation assessed by CD68 staining. The fact that Kupffer cells of recipient origin could be found in liver grafts, has been already known for many years but the use of combined FISH and immunohistochemistry allows a precise count of these cells; indeed, 69–100% of macrophages/Kupffer cells within liver allografts are recipient-derived cells and this proportion increases significantly with time reaching 95% at 10 months and beyond. Another recent study from Bittman et al. [17] is in agreement with these data but stresses upon the persistance of a smaller portion of donor Kupffer cells during the whole time course studied and the lack of correlation between the proportion of Kupffer cells of recipient origin and the histological alterations of the liver tissue or patient survival. Nevertheless it is likely that recipient-derived Kupffer cells may contribute to the immune tolerance of liver allografts. Liver sinusoidal endothelial cells are also involved in the local control of the immune response in the liver, and it has been shown that efficient presentation of exogenous antigen by liver endothelial cells to CD8 + cells results in antigen-specific T cell-tolerance [18]. In the present work $<1\%$ of sinusoidal endothelial cells in liver allografts are of recipient origin. This finding is not in agreement with the results of other studies [19,20] which indicate an extensive endothelial cell chimerism in liver allografts. It is noteworthy that similar endothelial cell chimerism is found in tolerant and non-tolerant liver transplant patients [20].

The presence of Y chromosome-positive cells within a female liver could also be interpreted as a consequence of feto-maternal chimerism. Fetal cells can be found in maternal blood and tissues for decades after childbirth and feto-maternal microchimerism in liver is a frequent phenomenon [21]; however it is a low-level phenomenon concerning $<1\%$ of all cells (personal data), so it is quantitatively negligible when compared to the level of recipient-derived chimerism in allografts as reported by Ng et al. [10]. But conversely from a qualitative point of view relying only on FISH, Johnson et al. obtained data supporting the fetal origin of male liver cells in the liver of a woman with chronic hepatitis C [22].

In summary, the publication from Ng et al. [10] yields a complete study both quantitative and qualitative of liver intragraft chimerism. The main points are the constant and high level of chimerism mainly due to the replacement of most donor Kupffer cells by recipient-derived cells and the minimal proportion of recipient-derived hepatocytes. After previous more optimistic reports, this last point could appear disappointing in view of cellular therapy for liver disease but may be 'functional and structural organization counts as much as quantity (if not more so)' [16].

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