Correspondence

Molecular Pathogenesis of Nonalcoholic Steatohepatitis

Today and Tomorrow

To the Editor-in-Chief:

Nonalcoholic fatty liver disease (NAFLD) is a growing hepatological problem in Western countries.¹ NAFLD may be limited to the fatty liver alone, or it may progress to nonalcoholic steatohepatitis (NASH). NASH is characterized by an association between inflammation and other liver lesions (eg, apoptosis, necrosis, and fibrosis) and may evolve into cirrhosis and hepatocellular carcinoma. Considerable progress has been made concerning the natural history of NASH from its first description in 1980 by Ludwig et al.² However, no therapy has yet been demonstrated efficacious in treating NASH patients. Furthermore, pathogenic mechanisms are the mainstay for therapeutic considerations. The most-recognized proposal for the pathogenesis of NASH is a “two-hit” hypothesis.³ Hepatic fat accumulation in the liver represents the “first hit,” which induces a “second hit” including insulin resistance, oxidative stress, and increased cytokine release. These modifications enhance lipid peroxidation, hepatocyte injury, and release of toxic byproducts, resulting in necroinflammation and fibrosis. Unfortunately, the study of the pathogenic factors involved in NASH is difficult because of the lack of a suitable experimental animal model.

We read with considerable interest the article by Otogawa et al⁴ published in the March issue of *The American Journal of Pathology*. In this article, the authors highlight that an increase of erythrophagocytosis induces iron accumulation in Kupffer cells, leading to some characteristic features of NASH such as necroinflammation, oxidative stress, and fibrosis. These considerations are supported by the reducing effect of phlebotomy on some parameters normally increased in the NASH model: serum levels of lipid peroxide and hydroxyproline, the number of proapoptotic liver cells, and the amounts of α -smooth muscle actin and deposited collagen.

As underlined by the authors, the major finding of their study is that they have developed a model that exhibits clinical, histological, and molecular features resembling human NASH. The multifactorial features observed in NASH patients are lacking in currently available animal

models, which are often rodents either with genetic defects (ob/ob mice or Fa/Fa rat) or fed a methionine- and choline-deficient diet.^{5,6} On the contrary, Otogawa et al⁴ use high-fat diet (HFD)-fed rabbits obtained with a standard diet supplemented with 20% corn oil and 1.25% (w/w) cholesterol, a very good model for pathogenetic study of NASH. In fact, after 8 weeks, HFD-fed rabbits display a significant increase in the liver weight relative to body weight, liver fat accumulation, cholesterol and triglyceride levels, serum and liver lipid peroxide levels, ratios between serum levels of immunoreactive insulin and blood sugar, liver activity of nuclear factor- κ B, and liver apoptotic rate. Moreover, in this report, the authors emphasize the central role of Kupffer cells in pathogenesis of NASH. This last evidence, surely, shows a strong correlation with the pattern of NASH patients, opening new ways of understanding the mechanisms leading to fibrosis. Thus, we suggest using HFD-fed rabbits for studying the correlation between histological liver injury scores and erythrophagocytosis by Kupffer cells. Concerning the relevance of this mechanism to progression from fatty liver to NASH, we believe that a more extensive study, based on cellular and molecular approaches, is required.

Anna Alisi
Valerio Nobili

*Children's Hospital and Research Institute “Bambino Gesù”
Rome, Italy*

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Authors' Reply:

We greatly appreciate Drs. Alisi and Nobili's comments on our recent work. NAFLD has become one of the critical chronic liver diseases worldwide. Approximately 5% of NAFLD is speculated to progress to NASH, and up to 12% of NASH patients progress to cirrhosis with life-threatening complications including hepatocellular carcinoma.^{1–4} Therefore, there is a great urgency to clarify the pathogenesis of NAFLD/NASH to establish reasonable treatment strategies for these diseases.

It has become gradually evident during the last few years that NAFLD is an intrinsic part of the so-called metabolic syndrome composed of obesity, impaired fasting glucose (type 2 diabetes), hyperlipidemia, and hypertension. In 1983, Buja et al⁵ reported an animal model of human familial hypercholesterolemia with developing arteriosclerosis using Watanabe heritable hyperlipidemic rabbits fed a rabbit chow supplemented with 2% cholesterol and 10% corn oil for 2 weeks. They also observed fatty accumulation in the liver of New Zealand White rabbits fed the HFD. Since then, the rabbit models have been used for the study of hypertension, hyperlipidemia, atherosclerosis, and hyperglycemia, that is, a metabolic syndrome. Here, it is important to understand the differences in lipid metabolism between animals.⁶ For instance, the main lipoproteins are high density lipoprotein and very low density lipoprotein in mice and rats, whereas it is low density lipoprotein in human and rabbits. Atherosclerosis develops relatively easily in humans and rabbits, but mice and rats are resistant. Furthermore, serum cholesterol level can be controlled by statins in humans and rabbits but not in mice or rats. In these regards, rabbits should be recognized as a much better experimental animal for developing a NAFLD/NASH model than mice and rats.

In the course of re-exploring the HFD-fed rabbits, we found that they exhibit steatohepatitis with fibrosis, implying that the model could be a new animal model of human NASH. NAFLD/NASH represents a modern illness basically caused by overnutrition of a high fat-containing diet, such as one containing many fast foods. Therefore, we

considered that a NASH model induced in mice and rats by a methionine- and choline-deficient diet is not a suitable animal model of a human NASH because it is unusual for modern humans to consume diets completely lacking these amino acids. Thus, we aimed to produce a better animal model of this disease reflecting modern lifestyle and dietary habits.

We found in this study that an increase of erythrophagocytosis by liver-resident macrophages, or Kupffer cells, contributes to the excess storage of iron in the steatotic liver, which may account, at least in part, for the initiation of hepatic inflammation and fibrosis due to the overproduction of free radicals. In fact, phlebotomy attenuated the accumulation of iron and the development of fibrosis in the liver of this model. In a human study, Facchini et al⁷ performed phlebotomy in NAFLD patients, resulting in improved serum insulin level and alanine aminotransferase.

In summary, our rabbit model of steatohepatitis serves as a desirable animal model of human NAFLD/NASH. More detailed analyses of the "two-hit theory" would be possible at gene expression and molecular levels in a time-dependent manner by using this model.

Kohji Otogawa
Norifumi Kawada

Osaka City University
Osaka, Japan

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