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Commentary

Liver inflammation and regeneration in drug-induced liver injury: sex matters!

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Drug-induced liver injury (DILI) remains a clinical challenge due to the poorly predictable outcomes. Accordingly, considerable efforts have been devoted to unravel the risk factors responsible for DILI worsening toward acute liver failure (ALF), liver transplantation (LT), and/or death. From a pathogenic point of view, exhaustion of drug metabolizing pathways, cell death mechanisms, activation of local immune cells, such as Kupffer cells, and recruitment of inflammatory leukocytes including monocytes and lymphocytes are key drivers of DILI progression. Taking into account that the liver is a sexually dimorphic organ, in the recent past several studies aimed to investigate the implications of gender differences in promoting DILI. While sex discrepancies in DILI include the hepatic drug metabolism or direct effects of steroid hormones (e.g. androgens and estrogens) on signaling pathways in the liver, relatively little is known on gender differences in modulating liver innate immune responses. In a previous issue of *Clinical Science*, Bizzaro and co-workers, analyzed sex-dependent differences in experimental acute liver injury and regeneration in mice. The authors observed a time-delay in the recovery process in male animals associated with a higher recruitment of monocytes expressing the androgen receptor (AR) as compared with females. Treatment of male mice with the pharmacological AR antagonist flutamide reduced monocyte recruitment in mice. Likewise, human male patients suffering from DILI displayed higher circulating immature and potentially more inflammatory monocytes. Altogether, these observations provide new insights into sex-dependent immune mechanisms in the context of acute liver injury, suggesting gender disparate inflammatory and regenerative responses following DILI.

Drug-induced liver injury (DILI) is a major hurdle in drug discovering and manufacturing, since it represents one of the most frequent reasons of failures in terms of marketing innovative therapeutic options [1]. DILI can manifest after the administration of a wide spectrum of medications and chemical compounds, such as antimicrobials and anticonvulsants, herbal remedies, and dietary supplements [1-3]. The manifestations of DILI are highly variable and dependent on the culprit drug. For clinicians, DILI is a challenging condition due to the poorly predictable outcomes. For instance, its idiosyncratic form (iDILI) might lead to acute liver failure (ALF) and ultimately to liver transplantation (LT) or death [2]. It has been noted from prospective clinical trials that female patients generally have a higher risk of adverse drug reactions than males, and some evidence suggests that women might be more susceptible than men to drug-related ALF as well as autoimmune hepatitis [4]. On the contrary, registry data and some prospective epidemiological studies did not confirm a gender imbalance in DILI cases [5-7]. Moreover, many confounding factors need to be considered that could influence sex-dependent differences in drug toxicity, including different alcohol drinking behavior, underlying fatty liver disease, autoimmunity, or adherence to prescribed medication.

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The liver is one of the most sexually dimorphic organs, and a gender disparity is basically observed in most types of liver injury, including autoimmune hepatitis, cholestatic disorders, non-alcoholic fatty liver

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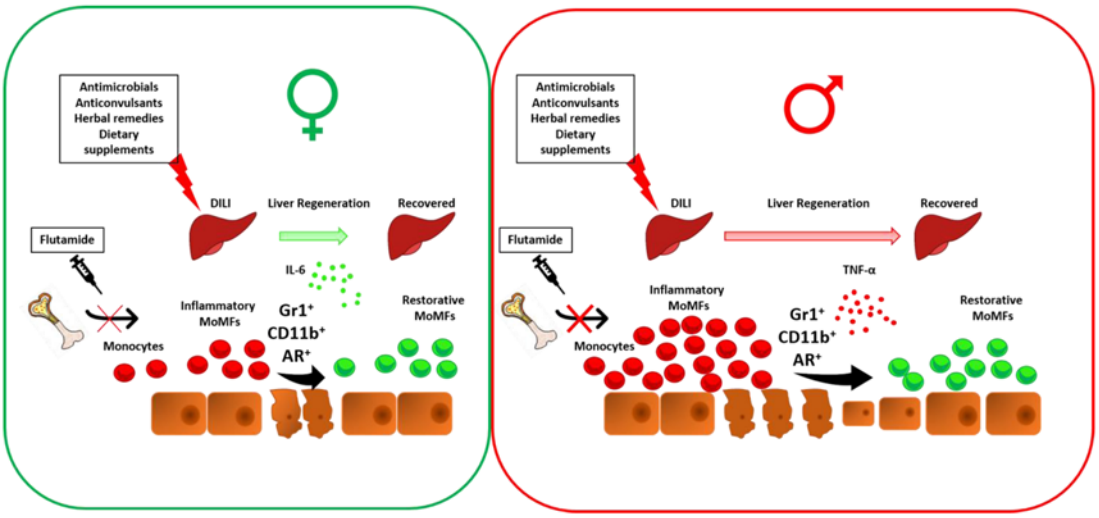

Figure 1. Sex-dependent differences in inflammatory responses and regeneration after acute liver injury

Female and male patients have a different susceptibility to drug-induced liver injury (DILI). In a previous issue of *Clinical Science*, Bizzaro and co-workers [17] investigated sex-dependent differences in a mouse model of CCl_4 injury. In this model, the liver is infiltrated by pro-inflammatory monocytes ($GR1^+CD11b^+$) expressing the androgen receptor (AR). Liver monocyte recruitment differed between genders in terms of kinetics, intensity, and cytokine milieu. In female mice, accumulation of monocyte-derived macropages (MoMF) was faster, but reduced, and associated with the expression of interleukin-6 (IL-6). On the contrary, in males, it was slower, greater and characterized by TNF-α production that promoted liver injury. Consequently, the restorative process appeared delayed in male compared with female mice. The administration of the AR antagonist flutamide reduced the hepatic recruitment of inflammatory $GR1^+CD11b^+AR^+$ monocytes efficiently in males.

disease, benign hepatic tumors, or liver cancer. Mechanisms underlying sex discrepancies in DILI include the hepatic metabolism of drugs as well as effects of steroid hormones such as androgens and estrogens on signaling pathways in the liver. For instance, the inhomogeneous expression of drug metabolizing enzymes and transporters deeply influence the pharmacokinetics, pharmacodynamics, and the appearance of side effects [8,9]. On the other hand, it is noteworthy that sexual hormones might also directly modulate hepatic inflammation by regulating the function of the immune system [10]. The mechanisms of sex differences in inflammatory and immune responses in the liver are not yet fully elucidated. In the mouse model of Concanavalin A-mediated acute hepatitis, standard operating procedures recommend to use only male animals due to the more pronounced and reproducible induction of the hepatic immune activation [11]. The presence of sexual dimorphism was also reported in cutaneous wound-healing [12], suggesting that regenerative and scarring responses are influenced by gender.

One of the key mechanisms for drug-related immune activation is the response of monocytes and macrophages to hepatic cell death [13]. Stress signals from injured hepatocytes, e.g. danger-associated molecular patterns (DAMPs) or alarmins, are recognized by locally surrounding Kupffer cells, resulting in the release of cytokines, chemokines, and the recruitment of leukocytes, amongst the monocytes, from the bloodstream into the liver [14]. Due to their high plasticity, pro-inflammatory monocytes can undergo a local reprogramming in the liver related to the cytokine milieu. When the injury has been resolved, monocytes can acquire a restorative phenotype and contribute to dampen inflammation, promote wound healing, and tissue macrophages replenishment [15,16]. It is currently unclear, whether and to which extent males or females differ with respect to macrophage activation, injury promotion, and injury resolution in the context of DILI.

In a previous issue of *Clinical Science*, Bizzaro and co-workers [17] comprehensively analyzed sex-dependent differences in acute liver injury and regeneration in BALB/c mice (Figure 1). They used acute poisoning with the hepatotoxic agent carbon tetrachloride (CCl_4), a model that has not been reported to be strikingly different between male and female animals [18], and focussed on three time points (i.e. 3, 5, and 8 days) during the restorative phase after CCl_4 injection. In this setting, they found a similar parenchymal injury in terms of necrotic areas between both genders, but conversely, male mice displayed higher ALT and AST release at 3 days after CCl_4. In addition, males showed a slower recovery capacity testified by the persistence of necrotic areas in the later phases, which was associated with the appearance of $CD68^+$ macrophage clusters. The authors tried to define which cell types were responsible for sex

dimorphism observed in liver healing rate. To unravel this issue, they evaluated the proliferative capability of hepatocytes along with the activation state of progenitor and stellate cells. Nonetheless, they did not find any meaningful differences by comparing genders [17].

Therefore, considering the increased $CD68^+$ macrophage numbers in the liver of male mice, they analyzed whether differences in immune system activation might account for the sex-dependent discrepancies seen in the recovery process. In support of this hypothesis, the authors found a significant up-regulation of TNF-α, interleukin (IL) 5 (IL-5), and IL-4 in males, conversely, the expression of IL-6, IFN-γ, IL-12β, and CXCL9 was more prominent in females. It is noteworthy that the hepatic abundance of IL-6 transcripts, a key driver of liver regeneration [19], was already higher in control females compared with males and further increased by liver damage. Moreover, in the same experimental model, they assayed the implication of sex hormones evaluating the hepatic expression of the androgen (AR) and estrogen (ER-α) receptors. Although a basal expression of AR has been reported in all control mice, it was significantly higher in males in steady state and further induced by CCl_4 injection. On the contrary, the authors did not observe any AR induction in livers of female mice upon injury. The ER-α expression, as expected, was enhanced in female livers at baseline, but not additionally stimulated during liver inflammation in both genders [17].

Mechanistically, the authors could link AR expression to the recruitment of inflammatory monocytes into the liver. While male mice had a higher overall hepatic macrophage accumulation after injury, the number of $CD11b^+F4/80^{high}$ monocyte-derived macrophages (MoMFs) was more prominent in the early phases of regeneration in females and appeared later in males. Moreover, liver MoMFs isolated by cell-sorting highlighted sex differences in their transcriptomic profile, including a higher AR and IL-5 expression in males and higher IL-6 in females. On a functional level, administration of flutamide, a pharmacological AR antagonist, strongly reduced the recruitment of $CD11b^{high}Gr1^{high}AR^+$ MoMF induced by CCl_4, suggesting that the AR is involved in liver monocytes attraction *in vivo* (Figure 1) [17]. These data support the relevance of sex for hepatic cyto-/chemokines expression [17], confirm the importance of pro-inflammatory monocytes in DILI [20] and support the role of AR in regulating monocyte/macrophages activation and recruitment in wound healing [21].

Importantly, the authors performed additional experiments analyzing sex differences in circulating monocytes from human patients with DILI. Although these observations are still preliminary due to the small number of studied subjects, DILI patients had a lower number of mature monocytes in peripheral blood compared with healthy subjects. Moreover, male patients had higher numbers of circulating monocytic progenitors ($CD33^+HLA\text{-}DR^+CD11b^-$) and promonocytes ($CD33^+HLA\text{-}DR^+CD11b^{\pm}$) than females, indicating a higher release of immature, potentially more inflammatory monocytes from the bone marrow into the bloodstream in men [17].

Taken together, the data reported by Bizzaro and co-workers [17] revealed sexual dimorphism in the pathogenesis and resolution of drug-induced liver damage through the modulation of hepatic monocyte recruitment by, to some extent, the AR. In addition, the pronounced presence of circulating immature monocytes in male DILI patients suggests that these mechanisms are relevant in the clinical setting of DILI. Nonetheless, the present study can only be the starting point of a thorough investigation in humans. At present, the relationship between immune responses, at the level of circulating monocytes or intrahepatic immune accumulation, and the outcome of DILI in male compared with female patients is not yet established. Nonetheless, these data might provide interesting starting points for new, gender-specific biomarkers such as the shedded monocyte receptor CD87 [22] or for novel therapeutic interventions targeting monocyte recruitment [23] or sex-hormone signaling [24]. Larger observational or prospective trials involving well-defined cohorts of patients with appropriate gender balance are needed to better understand sex-dependent immune mechanisms in DILI.

Before proposing novel therapeutic strategies, however, a series of additional preclinical work would be required. For instance, it would be important to exclude that the reported effects are model and/or strain dependent. To corroborate the interesting findings by Bizzaro and co-workers [17], additional models (e.g. acetaminophen poisoning) and genetic strains (e.g. the c57bl/6 background) should be analyzed, because interstrain variability can influence immune response polarization and ultimately the susceptibility to experimental liver disease [25]. Moreover, the current study demonstrated suppression of monocyte infiltration by flutamide, but provided no data on whether the AR antagonist affected the magnitude of the necrotic areas or ALT and AST release. In addition, monocytes have a dual function in DILI – disease promotion in early and tissue restoration in late phases [26]. Any type of targeted pharmacological therapy, such as AR antagonism, would need to balance potential effects on injury promotion compared with delayed resolution, either by dosing, timing of the intervention, or specificity of the targeted pathway. Certainly, further translational data from human patients during the course of DILI could help to design such novel therapeutic strategies. In the era of personalized medicine, however, sex differences in inflammation and immunity should not be neglected [24].

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The observations described by Bizzaro and co-workers [17] might be very well relevant beyond acute hepatitis and DILI. A similar gender disparity has been described for incidence of hepatocellular carcinoma (HCC). Men display a higher susceptibility for developing HCC compared with women, and such differences can be well recapitulated in rodents exposed to the liver carcinogen diethylnitrosamine (DEN) [27,28]. In the last decades, several studies dissected molecular mechanisms underlying this sexual dimorphism indicating a fostering role for androgens and a protective effect for estrogens. Interestingly, the beneficial action of estrogens is associated with their capacity to dampen IL-6 secretion by Kupffer cells [29]. This fits to the role of IL-6 as a hepatocyte mitogen related to liver regeneration and tumorigenesis [30], but is contrary to the observations by Bizzaro and co-workers [17] in their experimental model. Another aspect of sex-dependent differences in liver diseases relates to non-alcoholic steatohepatitis (NASH). Male sex is considered a risk factor for disease progression in NASH [31]. This has been mainly linked to the role of estrogen for hepatic lipid metabolism [32] and to the role of visceral adipose tissue for NASH [33], but not convincingly to sex differences in macrophage activation or monocyte recruitment [34]. Thus, the current study reminds us that 'the little difference' can have huge consequences, even for evolutionary conserved processes such as drug-induced hepatotoxicity, inflammation, and liver regeneration.

Competing interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

ALF, acute liver failure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AR, androgen receptor; CXCL9, CXC motif chemokine ligand 9; DILI, drug-induced liver injury; ER-α, estrogen receptor; HCC, hepatocellular carcinoma; IFN, interferon; IL, interleukin; MoMF, monocyte-derived macrophage; NASH, non-alcoholic steatohepatitis; TNF, tumor necrosis factor.

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