ORIGINAL ARTICLE

High-density lipoprotein infusion protects from acute graft-versus-host disease in experimental allogeneic hematopoietic cell transplantation

Cécile Chagué[1] | Thomas Gautier[2] | Ludivine Dal Zuffo[1] | Jean-Paul Pais de Barros[2] | Audrey Wetzel[1] | Georges Tarris[1,3] | Gaëtan Pallot[2] | Laurent Martin[1,3] | Séverine Valmary-Degano[4] | Valérie Deckert[2] | Laurent Lagrost[2] | Etienne Daguindau[1,5] | Philippe Saas[1]

[1]University Bourgogne Franche-Comté, INSERM, EFS BFC, UMR1098 RIGHT Interactions Greffon-Hôte-Tumeur/Ingénierie Cellulaire et Génique, LabEX LipSTIC, FHU INCREASE, Besançon, France

[2]University Bourgogne Franche-Comté, INSERM, LNC UMR1231, LabEX LipSTIC, Dijon, France

[3]Service d'Anatomie et Cytologie Pathologiques, CHU Dijon, Dijon, France

[4]Service d'Anatomie et Cytologie Pathologiques, CHU Besançon, Besançon, France

[5]Service d'Hématologie, CHU Besançon, Besançon, France

Correspondence
Philippe Saas, University Bourgogne Franche-Comté, INSERM, EFS BFC, UMR1098 RIGHT Interactions Greffon-Hôte-Tumeur/Ingénierie Cellulaire et Génique, LabEX LipSTIC, FHU INCREASE, 8 rue du Dr JFX Girod, F-25000 Besançon, France.
Email: philippe.saas@efs.sante.fr

Funding information
This work is supported by the Agence Nationale de la Recherche (ANR) under the program "Investissements d'Avenir" (ANR-11-LABX-0021-LipSTIC), by the Région Bourgogne Franche-Comté, by the MiMedI project funded by BPI France (grant no. DOS0060162/00) and the European Union through the European Regional Development Fund of the Region Bourgogne Franche-Comte (grant no. FC0013440), as well as by the Institut Carnot OPALE.

Acute graft-versus-host disease (aGVHD) is a major limitation of the therapeutic potential of allogeneic hematopoietic cell transplantation. Lipopolysaccharides (LPS) derived from intestinal gram-negative bacteria are well-known aGVHD triggers and amplifiers. Here, we explored the LPS metabolism in aGVHD mouse models using an innovative quantification method. We demonstrated that systemic LPS accumulation after transplantation was due, at least partly, to a defect in its clearance through lipoprotein-mediated transport to the liver (i.e., the so-called reverse LPS transport). After transplantation, reduced circulating HDL concentration impaired LPS neutralization and elimination through biliary flux. Accordingly, HDL-deficient (*Apoa1*^{tm1Unc}) recipient mice developed exacerbated aGVHD. Repeated administration of HDL isolated from human plasma significantly decreased the mortality and the severity of aGVHD. While the potential role of HDL in scavenging circulating LPS was examined in this study, it appears that HDL plays a more direct immunomodulatory role by limiting or controlling aGVHD. Notably, HDL infusion mitigated liver aGVHD by diminishing immune infiltration (e.g., interferon-γ-secreting CD8$^+$ T cells and non-resident

- -

macrophages), systemic and local inflammation (notably cholangitis). Hence, our results revealed the interest of HDL-based therapies in the prevention of aGVHD.

1 | INTRODUCTION

Allogeneic hematopoietic cell transplantation (alloHCT) is the only curative therapy for some hematologic malignancies. Its efficacy is dampened by acute graft-versus-host disease (aGVHD), a severe inflammatory reaction associated with important mortality and morbidity rates. It has been suspected that preliminary recipient conditioning alters the intestinal barrier integrity by inducing intestinal epithelial cell (IEC) apoptosis, promoting immune cell infiltration, and disorganizing the crypt/villus structures.[1] Recently, it has been proposed that the conditioning regimen combined with early allogeneic immune responses alters type 2 innate lymphoid cells (ILCs) inducing damage-associated molecular pattern release and local tolerance inhibition.[2] Allogeneic donor T cells then reach intestinal epithelium and create tissue damages by injuring Paneth cells, type 3 ILC and IEC.[3] Intestinal epithelium damages compromise the barrier function and act as a permissive mechanism in intestinal aGVHD.[4] At the same time, antibiotic exposure and antimicrobial peptide deregulation can lead to dysbiosis (i.e., alteration of microbiota diversity) and facilitate pathogenic microorganism emergence, such as gram-negative bacteria.[5,6] These bacteria and their metabolites can cross the injured epithelium from the intestinal lumen to general circulation by bacterial translocation. Although some metabolites from commensal bacteria, such as butyrate[7] or indoles,[8,9] have protective effects on intestinal barrier, other microbiota-derived compounds can trigger inflammation. Lipopolysaccharides (LPS), major outer membrane components released by gram-negative bacteria during their division or lysis, are well-described aGVHD inducers. The presence of LPS in the systemic circulation triggers recipient antigen-presenting cell (APC) priming, cytokine release and mucosal damage sustainment, leading to a durable inflammatory state.[1,10,11] Interruption of LPS inflammatory signaling by genetic[12] or pharmacological[13] inhibition, protects recipients from aGVHD in alloHCT mouse models.

Nevertheless, none of these studies has been translated to a clinical therapeutic approach. For this reason, we hypothesized that a comprehensive analysis of LPS metabolism in the course of aGVHD could bring into light new prophylaxis or treatment strategies.[14] The use of innovative LPS quantification techniques allows the exploration of the reverse LPS transport (RLT). The RLT, initially described in sepsis,[15] corresponds to the neutralization and elimination of LPS by circulating lipoproteins. This pathway was named after its similarities with reverse cholesterol transport in which cholesterol in excess in extrahepatic compartments is integrated into circulating lipoproteins for liver clearance.[15,16] LPS are amphipathic molecules harboring a hydrophilic, polysaccharidic moiety (O antigen and core) and a hydrophobic domain (lipid A) containing several acyl chains. This latter moiety is anchored in bacterial membranes and carries the immune stimulatory capacities of the molecule. Because LPS partly share their structure with host endogenous lipids,[16] they can be incorporated in high-density lipoproteins (HDLs) by plasmatic phospholipid transfer protein (PLTP) or LPS-binding protein (LBP) activities,[17] or by CD14 transfer.[18] The binding of LPS to HDL masks the lipid A moiety, thus limiting its activity, and facilitates its transport to the liver. HDL then deliver LPS to hepatocytes, endothelial cells, and Küpffer cells (i.e., hepatic resident macrophages)[19,20] for detoxification and biliary elimination.[21,22]

In this study, using mouse alloHCT models, we confirmed the systemic LPS exposure by an innovative technique based on high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC/MS/MS) (Endoquant®[23]) and investigated the role of RLT by modulating several effectors. Our major findings showed that in the complete absence of circulating HDL (i.e., in Apoa1[tm1Unc] mice), recipient mice experienced a more severe aGVHD associated with an increased APC maturation and type 1 T cell responses in the spleen and the liver. Furthermore, the level of circulating HDL was collapsed in aGVHD mice but was corrected by repeated intravenous infusion of exogenous HDL. The restoration of HDL pool led to an increased survival and a decreased aGVHD severity. HDL infusion reduced the concentration of circulating and biliary LPS, the production of pro-inflammatory cytokines by liver macrophages, as well as limits liver alloreactive Tc1 responses and injuries. Overall, these results suggest that HDL infusion is a potential prophylactic approach for aGVHD.

## 2 | MATERIALS AND METHODS

Please refer to the Supplementary information.

3 | RESULTS

3.1 | Allogeneic T cell infusion lowers LPS neutralizing capacity of plasma due to a circulating HDL drop

Due to their heterogeneity and the lability of their physicochemical properties, the quantification of LPS, notably in biological fluids, has always been tricky. Previous studies on the impact of LPS during

aGVHD used the *Limulus* amebocyte lysate (LAL) assay.[10,11] This approach only reflects free and biologically active LPS. In order to characterize the LPS metabolism in the C57Bl/6 → BALB/c mouse aGVHD model (Figure 1A, Figure S2), we measured 3HM, the most common hydroxylated fatty acid found in LPS lipid A, using HPLC/
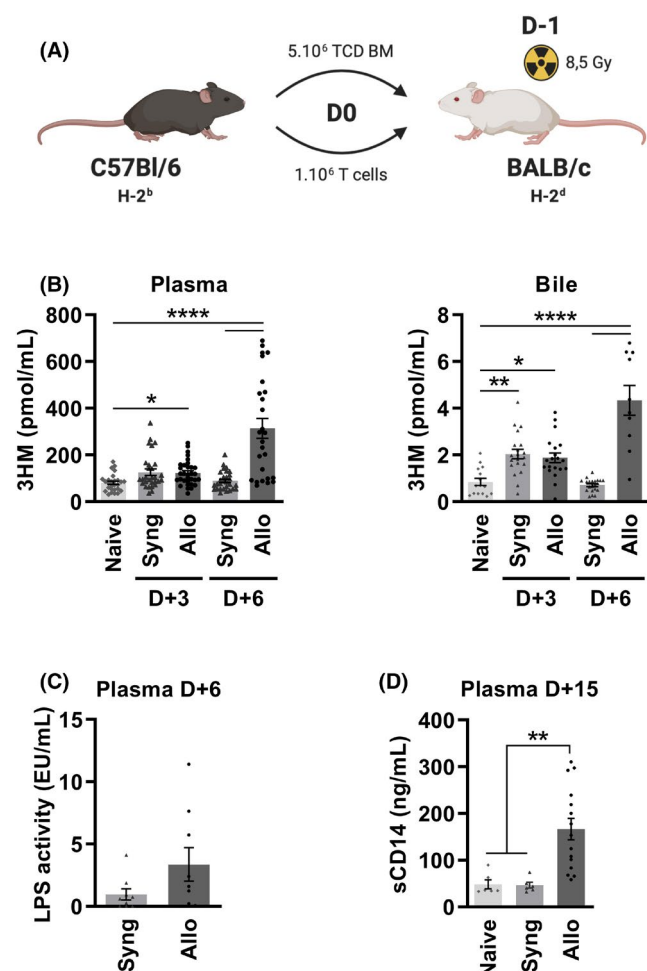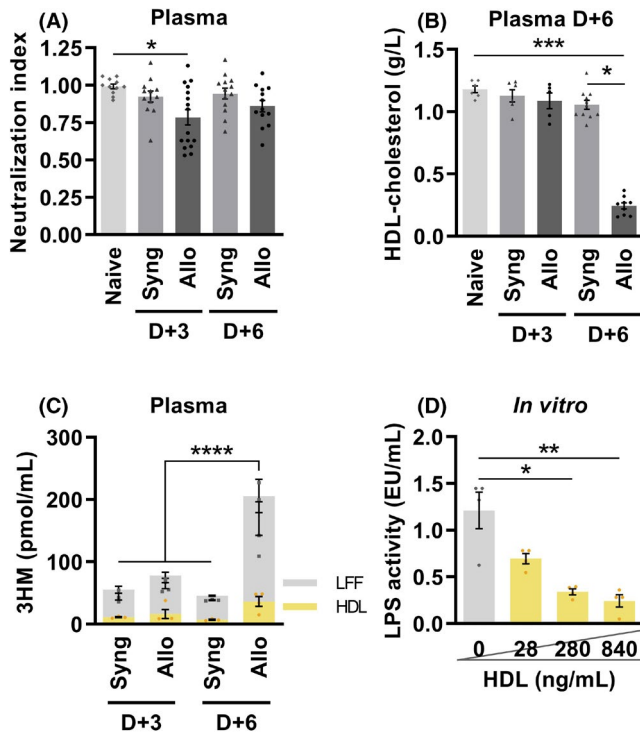

FIGURE 1 Allogeneic T cell infusion induces early LPS translocation. (A) Lethally irradiated BALB/c recipients (8.5 Gy) were grafted with 5×10^6 T cell–depleted bone marrow (TCD BM) and 1×10^6 splenic T cells from BALB/c (Syng) or C57Bl/6 (Allo) donors (C57Bl/6 → BALB/c model). (B) 3-hydroxymyristic acid (3HM), the most common hydroxylated fatty acid found in LPS lipid A, was quantified in the plasma and bile of recipient mice 3 and 6 days after BMT using HPLC/MS/MS technology (Plasma: $n = 26$–33 mice/group from six independent experiments, Kruskal-Wallis and Dunn's post-test; Bile: $n = 10$–21 mice/group from three independent experiments, one-way ANOVA and Bonferroni's post-test). (C) Activity of circulating LPS was assessed by *Limulus* amebocyte lysate (LAL) assay at day+6 after BMT and tended to show a rise in biologically active LPS in the plasma of the allo-transplanted mice ($n = 9$ mice/group from three independent experiments, Mann-Whitney test). (D) The soluble form of TLR4/MD-2 co-receptor CD14 (sCD14) concentration was assessed in the plasma of allo-recipient mice at day+15 after transplantation by ELISA ($n = 6$–15 mice/group from three independent experiments, Kruskal-Wallis and Dunn's post-test) (*$p < .05$, **$p < .01$, ****$p < .0001$)

MS/MS[23] (Figure 1B). Six days after transplantation, plasmatic and biliary 3HM was significantly higher in allografted mice compared to syngeneic controls. This was confirmed by a trend toward a three-fold increase in the plasmatic LPS activity measured by LAL assay (Figure 1C). This phenomenon was sustained by the rise of soluble CD14, a toll-like receptor 4 (TLR4) cofactor and nonspecific marker of monocyte activation, in the allografted mouse plasma at day+15 after transplantation (Figure 1D). This could reflect an extended LPS exposure. Previous studies demonstrated that systemic LPS came mainly from intestinal bacterial translocation,[1,5] and we confirmed the intestinal epithelium disruption in our model (Figure S2).

In physiological conditions, systemic LPS would be neutralized and eliminated by the RLT. As LPS accumulate in the plasma of recipient mice, we hypothesized that RLT might be impaired in the course of aGVHD. Using a HEK-Blue TLR4 cell-based test, we noticed a decreased ability of allografted mouse plasma to neutralize the activity of a known amount of *Escherichia coli* LPS in the first days following transplantation (Figure 2A). These data suggest that one or more plasmatic compounds failed to achieve LPS activity neutralization. In sepsis, it has been demonstrated that PLTP activity, through its role of LPS transfer during RLT, is critical and that recombinant PLTP administration reduces sepsis-induced mortality in mouse models.[24] Although PLTP activity was slightly decreased after allogeneic T cell infusion, the complete lack of PLTP activity in $pltp^{-/-}$ recipient did not exacerbate aGVHD (Figure S3). Hence, PLTP activity appears as a potential factor in the impairment of RLT during aGVHD, but not a critical one. We next presumed that the transporter particles themselves could be the limiting factor. In mice, LPS are mainly transported by HDL which are the major lipoproteins in these mammals.[25] We observed a collapse in circulating HDL levels as soon as day+6 post-transplantation in plasma from recipient mice (Figure 2B). While LPS associated with HDL represented an average of 16.5% of total circulating LPS in both allogeneic and syngeneic mice, the concentration of LPS in the lipoprotein free fraction was more than four times higher in allotransplanted recipients (Figure 2C). Consequently, it seems that the RLT capacity is saturated in our experimental aGVHD model. To assess if the LPS neutralizing capacity relies on the availability of HDL, increasing doses of HDL isolated from healthy blood donors were incubated with 1 EU/ml *E. coli* LPS and the remaining detectable LPS activity was measured with HEK-Blue TLR4 test (Figure 2D). We confirmed in vitro that LPS activity neutralization by HDL particles is a dose-dependent mechanism and reinforced the hypothesis that HDL is critical in LPS metabolism in the course of aGVHD.

3.2 | The loss of apolipoprotein synthesis and of circulating HDL increases aGVHD severity by worsening immune cell infiltration and favoring pro-inflammatory phenotypes in the liver

To confirm the impact of circulating HDL on the severity of aGVHD, we developed a C3H → C57Bl/6 mouse model using wild type (WT)
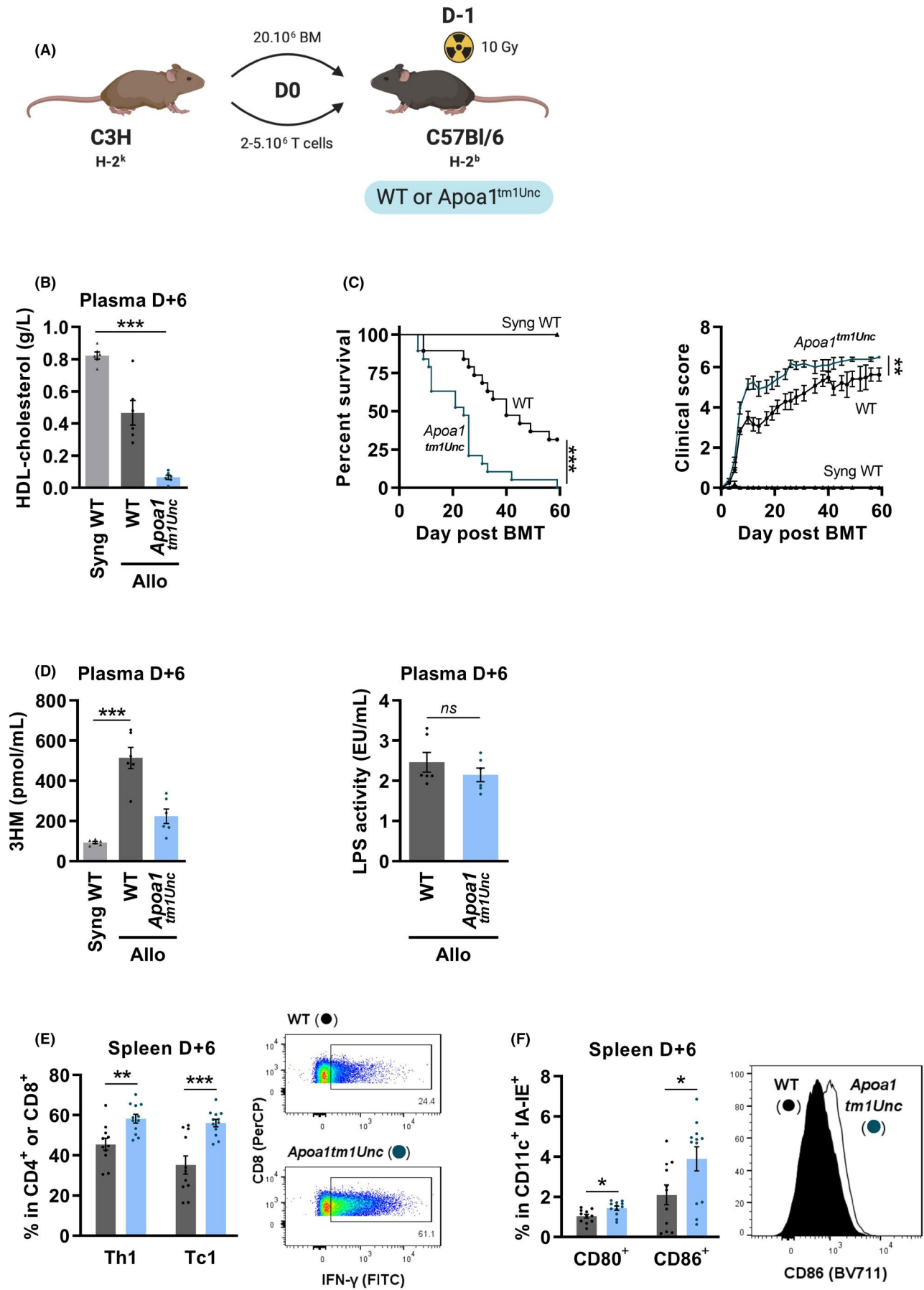
FIGURE 2 The systemic LPS rise observed after allogeneic T cell infusion is associated with a lower capacity to neutralize LPS due to a drop of circulating HDL. (A) The capacity of C57Bl/6 → BALB/c recipient mouse plasma to neutralize *E. coli* O55:B5 LPS activity was estimated in vitro using HEK-Blue TLR4 cells. The neutralization index was calculated as the ratio between the percentages of LPS neutralized by a recipient mouse plasma sample and a naive mouse sample ($n = 12–16$ mice/group from two independent experiments, Kruskal-Wallis and Dunn's post-test). (B) Circulating HDL-cholesterol level was quantified in the plasma of C57Bl/6 → BALB/c recipients using a ThermoFischer Indiko analyzer ($n = 5–11$ mice/group from two independent experiments, Kruskal-Wallis and Dunn's post-test). (C) The plasma of recipient mice was submitted to sequential ultracentrifugations in order to isolate a lipoprotein free fraction (LFF, $d > 1.21$) and an HDL-bounded fraction (HDL, $1.063 < d < 1.21$). 3HM quantitation in each fraction using Endoquant® technology revealed that almost all the extra LPS found in the allogeneic group was located in the LFF ($n = 3–4$ mice, two-way ANOVA and Bonferroni's post-test). (D) The ability of increasing doses of HDL isolated from healthy human plasma to neutralize *E. coli* O55:B5 LPS activity was analyzed using HEK-Blue TLR4 cells ($n = $ four independent experiments, Friedman and Dunn's post-test) (*$p < .05$, **$p < .01$, ***$p < .001$, ****$p < .0001$)

or apolipoprotein A-I (ApoA-I) deficient (*Apoa1^{tmc1Unc}*) recipients (Figure 3A). HDL-cholesterol measurements confirmed: (i) the collapse of circulating HDL after alloHCT in a second mouse model; and (ii) the barely detectable circulating HDL concentration in *Apoa1^{tmc1Unc}* recipients (0.07 ± 0.01 g/L) (Figure 3B). *Apoa1^{tmc1Unc}* mice experienced significantly increased mortality and clinical score after allogeneic T cell infusion (Figure 3C), suggesting a high impact of HDL on the physiopathology of aGVHD. The *Apoa1^{tmc1Unc}* knockout did not affect survival or general health of mice grafted with T cell–depleted bone marrow (TCD BM) only (Figure S4). Basal phenotype and in vitro polarization of splenic T cells were not affected by the *Apoa1^{tm1Unc}* genotype (Figure S5). Thus, the poor survival of *Apoa1^{tmc1Unc}* allografted mice is unlikely explained by a higher radiation sensitivity or a particular polarization pattern of this strain. The quantification of 3HM by Endoquant® at day+6 after transplantation (Figure 3D) revealed reduced amount of LPS in the plasma of *Apoa1^{tmc1Unc}* mice compared to their WT counterparts, while this concentration remained superior to those of syngeneic controls. However, the LAL assay showed a similar plasmatic LPS activity in both *Apoa1^{tmc1Unc}* and WT recipients. This suggests that a part of circulating LPS was still neutralized by the remaining HDL in WT mice, whereas all the LPS stood in a free form in *Apoa1^{tmc1Unc}* plasma. Acute GVHD in the C3H → C57Bl/6 model is known to be less severe than in the C57Bl/6 → BALB/c model; we observed a less important decrease in circulating HDL levels. Nonetheless, the lack of 3HM elimination in *Apoa1^{tmc1Unc}* recipient mice appears to be insufficient to explain the worsened GVHD severity. Therefore, we next investigated the immunological impacts of the loss of apolipoprotein synthesis and of circulating HDL. In the spleen, the proportions of both CD4+ and CD8+ T cells producing IFN-γ (T helper 1 [Th1] and T cytotoxic 1 [Tc1] cells, respectively) were significantly increased in *Apoa1^{tmc1Unc}* mice 6 days after the transplantation (Figure 3E, Figure S6). At the same time, a higher expression of CD80 and CD86 was detected on CD11c+IA/IE+ splenic dendritic cells (DC) of the transgenic recipients (Figure 3F, Figure S7). Altogether, these results document the deleterious outcomes of circulating HDL loss on the survival and the severity of aGVHD, resulting in an amplified Th1/Tc1 polarization and in an accelerated maturation of DC in the spleen.

While sacrificing the mice at day+6 post-transplantation, we noticed macroscopic changes of *Apoa1^{tmc1Unc}* mouse liver aspect, with a size enlargement and a poorer perfusion. As a target organ

FIGURE 3 The loss of apolipoprotein synthesis and of circulating HDL increases the severity of GVHD. (A) Lethally irradiated C57Bl/6 recipients (10 Gy) expressing (WT) or not (*Apoa1^{tmc1Unc}*) the gene of the apolipoprotein A-I (ApoA-I) were grafted with 20×10^6 bone marrow cells and $2-5 \times 10^6$ splenic T cells from C57Bl/6 (Syng) or C3H (Allo) donors (C3H → C57Bl/6 model). (B) Circulating HDL-cholesterol level was quantified in the plasma of recipient mice using a ThermoFischer Indiko analyzer ($n = 6$ mice/group from two independent experiments, Kruskal-Wallis and Dunn's post-test). (C) Mortality and clinical score of *Apoa1^{tmc1Unc}* recipients were significantly increased compared to the WT recipients ($n = 3–19$ mice/group from three independent experiments, log-rank test or Kruskal-Wallis and Dunn's post-test on AUC). (D) At day+6 after BMT, the plasmatic concentration of 3HM was assessed by Endoquant® method and the LPS activity was quantified using LAL assay ($n = 6$ from two independent experiments, Kruskal-Wallis and Dunn's post-test or Mann-Whitney test). (E) Proportions of CD3+CD4+ (Th1) and CD3+CD8+ (Tc1) T cells secreting IFN-γ after a 4h-PMA/ionomycin stimulation and (F) expression of maturation markers CD80 and CD86 on DC surface were analyzed in the spleen of *Apoa1^{tmc1Unc}* or WT recipients by flow cytometry ($n = 11–12$ mice/group from three independent experiments, unpaired *t*-test with Welch's correction) (*$p < .05$, **$p < .01$, ***$p < .001$)

(A)

20.10⁶ BM

D0

2-5.10⁶ T cells

C3H
H-2ᵏ

D-1

10 Gy

C57Bl/6
H-2ᵇ

WT or Apoa1^tm1Unc

(B) Plasma D+6

HDL-cholesterol (g/L)

Syng WT / WT / Apoa1^tm1Unc
Allo

***

(C)

Percent survival vs Day post BMT

Syng WT / WT / Apoa1^tm1Unc

***

Clinical score vs Day post BMT

Apoa1^tm1Unc / WT / Syng WT

**

(D) Plasma D+6

3HM (pmol/mL)

Syng WT / WT / Apoa1^tm1Unc
Allo

***

Plasma D+6

LPS activity (EU/mL)

WT / Apoa1^tm1Unc
Allo

ns

(E) Spleen D+6

% in CD4⁺ or CD8⁺

Th1 / Tc1

** / ***

WT (●)

CD8 (PerCP) vs IFN-γ (FITC)

24.4

Apoa1tm1Unc (●)

61.1

(F) Spleen D+6

% in CD11c⁺ IA-IE⁺

CD80⁺ / CD86⁺

* / *

WT (●) / Apoa1 tm1Unc (●)

CD86 (BV711)

of aGVHD,[26] the main ApoA-I producer[27] as well as the place of LPS elimination, liver may play a central role in LPS metabolism after alloHCT. To better understand the role of liver in relationship with ApoA-I and HDL deficiency during aGVHD, the infiltrating immune cells were analyzed by flow cytometry. An increase in CD45^{+} cells in *Apoa1[tmc1Unc]* recipient liver ($4.65 \pm 0.65 \times 10^6$ cells) compared to WT allografted mice ($2.80 \pm 0.20 \times 10^6$ cells) was observed (Figure 4A). As in the spleen, the proportions of hepatic Th1 and Tc1 cells rose in allografted *Apoa1[tmc1Unc]* mice (Figure 4B, Figure S6). Then, we analyzed two subclasses of liver macrophages: CD11b^{+}F4/80^{low} Küpffer cells (resident macrophages) and CD11b^{+}F4/80^{high} NRM derived from monocytes, as described.[28] The number of infiltrating NRM was nearly doubled in the liver of allografted mice lacking circulating HDL ($2.24 \pm 0.38 \times 10^5$ cells) compared to their WT counterparts ($1.21 \pm 0.14 \times 10^5$ cells) (Figure 4C). The number of Küpffer cells trended to rise the same way. Furthermore, IL-6 (Figure 4D) and TNF-α (Figure 4E, Figure S8) secretion of both Küpffer cells and NRM were significantly enhanced in *Apoa1[tmc1Unc]* recipients after ex vivo LPS stimulation. Altogether, this demonstrates that the absence of ApoA-I synthesis and of circulating HDL aggravates hepatic aGVHD and worsens mortality of allogeneic recipients.

3.3 | HDL infusion reduces aGVHD severity by partially neutralizing available LPS and lowering local inflammation in the liver

After confirming that alteration of HDL metabolism was a critical element in the aGVHD physiopathology, we proposed a therapeutic approach aiming to restore the circulating HDL level in recipient mice. In the C57Bl/6 → BALB/c model, mice received 12 intravenous infusions of HDL isolated from plasma of healthy donors (20 mg/kg) between day-1 and day+24 after transplantation (Figure 5A). This administration schedule raised significantly HDL-cholesterol levels in recipient plasma as early as day+6 after alloHCT (0.56 ± 0.10 g/L for treated mice vs. 0.31 ± 0.03 g/L for mice that received only NaCl) (Figure 5B), without completely recovering the syngeneic group level (1.06 ± 0.04 g/L). HDL infusion limited the long-term severity of aGVHD by doubling the median survival time (45 vs. 22 days) and mitigating clinical score (Figure 5C). Early after transplantation, systemic total LPS measured by both Endoquant® and LAL assay tended to decrease in the plasma and in the bile of HDL-treated mice (Figure 5D). Even if these results were not statistically significant, 3HM was reduced on average by 28.6% in the plasma of treated mice; this decline in both compartments was inversely correlated with the circulating HDL level with Spearman $r = -0.6897$ in plasma ($p < .0001$, Figure 5D) and $r = -0.7912$ in the bile ($p < .0001$, data not shown) of allografted mice. We assumed here that, at this early time-point, LPS were taken up by HDL, its activity was neutralized but it had not been eliminated yet through the liver. Regarding immunological responses, circulating IL-6 (Figure 5E) and ex vivo proliferation of naive T cells cultured with recipient splenocytes were decreased for HDL-treated mice (Figure S9).

Since the liver plays a major role in the occurrence of aGVHD,[30] we compared the infiltrating immune cells of mice treated or not by HDL using flow cytometry. Early after transplantation, the number of cells was slightly decreased by the HDL treatment (Figure 6A). At the same time, the proportion of CD8^{+} cells among T cells decreased when mice received HDL infusions. Moreover, the number of Tc1 cells was also significantly reduced (Figure 6B, Figure S6). In contrast, we did not observe any change in the number or the proportion of IL-17^{+} CD4^{+} and IL-17^{+} CD8^{+} T cells in the liver of HDL-treated mice (Figure S10). In the first days after alloHCT, the HDL treatment modulated mainly hepatic NRM. Notably, NRM from allografted mice treated with HDL exhibited a significantly lower production of IL-12 than their control counterparts (Figure 6C, Figure S8). The effect of HDL treatment was sustained in the course of aGVHD, as the trend toward reduced liver immune infiltrate persisted at the end of the infusion period (day+24, $9.53 \pm 1.17 \times 10^6$ vs. $11.78 \pm 0.94 \times 10^6$ cells) (Figure 6A). Of note, at this time-point, while HDL-treated mice exhibited a 75% survival rate, only 40% of mice receiving vehicle were still alive (Figure 5C). At day+24 after transplantation, HDL treatment reduced the cytokine production of Küpffer cells after ex vivo LPS stimulation with a significant decrease in IL-12 (Figure 6D, Figure S8). Finally, histological analysis of the liver at day+24 post-transplantation revealed that HDL-treated mice exhibited less cholangitis than untreated mice (Figure 6E). Therefore, the repeated administration of HDL permitted to rise up the level of circulating HDL of recipient mice (Figure 5B). Consequently, the mice were protected from aGVHD-related mortality and experienced a less severe form of the disease. The mechanisms involved in this protection could imply, at least partly, the reduction of circulating active LPS concentration and the limitation of hepatic inflammation and of Tc1 infiltration, leading to a mitigated liver aGVHD.

4 | DISCUSSION

Acute GVHD remains a major limitation of alloHCT as it occurs in nearly half of the recipients.[31] Its first-line glucosteroid treatment is effective only for 40%–70% of the cases depending on its severity.[32] The use of ruxolitinib constitutes a recent significant advance to treat steroid-refractory aGVHD (SR-aGVHD).[33] Nonetheless, SR-aGVHD remains a serious and difficult-to-treat entity. The development of original therapeutic approaches is needed to improve alloHCT outcomes and widen transplantation indications. New strategies are promising, such as fecal microbiota transplantation.[34,35] This approach derived from increasing knowledge about intestinal microbiota impacts on alloHCT outcomes. Recently, motifs of intestinal microbiota modifications in allografted patients were identified as reproducible toward different transplantation centers.[6] Even though the causal link between dysbiosis and aGVHD remains unclear, some studies have pointed out the impact of microbiota-derived metabolites in the severity and mortality associated with aGVHD.[7,9]
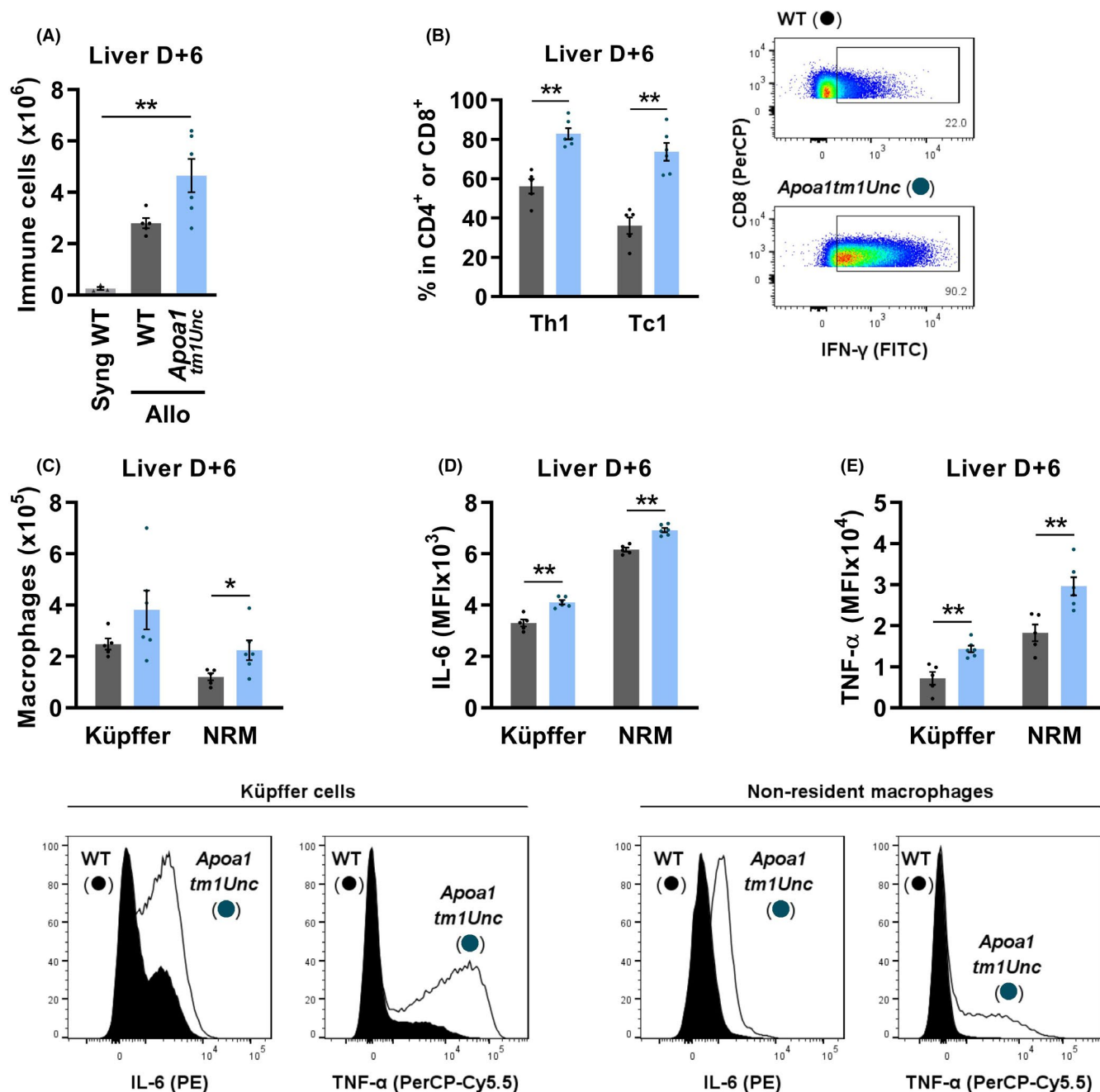
FIGURE 4 The loss of apolipoprotein synthesis and of circulating HDL worsens immune cell infiltration and favors their pro-inflammatory phenotype in the liver. (A) Six days after transplantation, immune cells were quantified in the mouse liver after an isolation using a Percoll gradient[29] (n = 4–6 mice/group, Kruskal-Wallis and Dunn's post-test). (B) T cell polarization, notably the proportions of CD4+ (Th1) and CD8+ (Tc1) T cells secreting IFN-γ, was analyzed in the mouse liver by flow cytometry on day+6 after BMT (n = 5–6 mice/group, Mann-Whitney test). (C) Hepatic macrophages were distinguished as resident Küpffer cells (CD11b+F4/80high) and non-resident macrophages (NRM, CD11b+F4/80low)[28] (n = 5–6 mice/group, Mann-Whitney test). (D,E) IL-6 and TNF-α secretion of Küpffer cells and NRM was quantified by intracellular staining analysis using flow cytometry after a 4h-LPS E. coli O55:B5 stimulation (n = 5–6 mice/group, Mann-Whitney test) (** p < .01)

Along with dysbiosis, intestinal damages allow the translocation of microorganisms, notably pathogenic bacteria, and their metabolites from the intestinal lumen to the general circulation. One of the first and most documented examples is LPS, released from gram-negative bacteria. As a danger signal, it strongly activates immune cells and LPS leakage has already been described in aGVHD setting.[1,10,11] However, these studies used mainly the LAL assay, which did not depict the whole picture of LPS quantification. For this reason, we decided to quantify LPS and to explore its metabolism, beyond its presence in circulation, with a HPLC/MS/MS method[23] that allows us to detect total LPS. Our major findings confirm the rise of systemic LPS in mouse models of alloHCT. This is associated
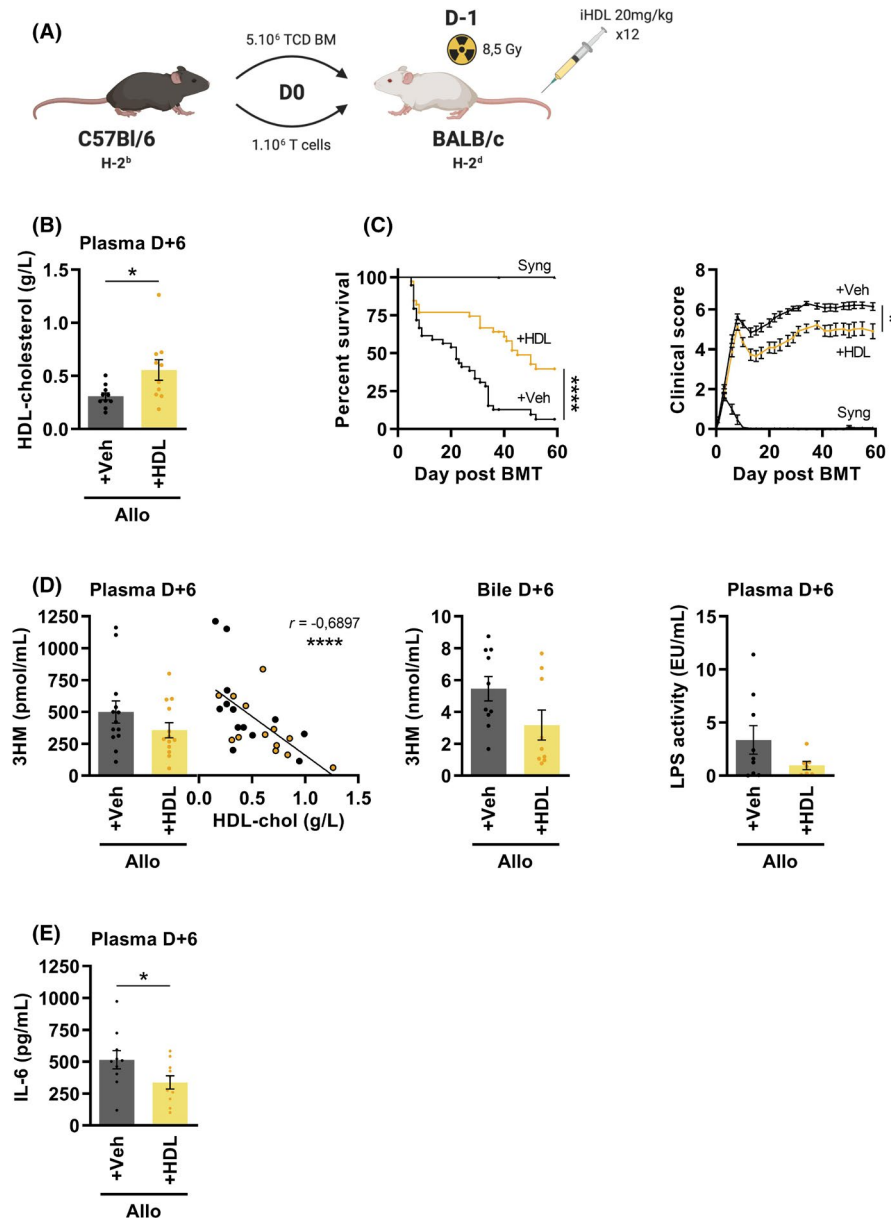
FIGURE 5 HDL infusion reduces GVHD severity and partially neutralizes available LPS. (A) Recipient mice from the C57Bl/6 → BALB/c model were treated three times a week by intravenous injections of HDL isolated from human plasma (20mg/kg, +HDL) or by vehicle (NaCl, +Veh) between D-1 and D+24 after BMT. (B) The circulating HDL-cholesterol level was measured with a ThermoFischer Indiko analyzer in the plasma of recipient mice 6 days after transplantation ($n = 10$ mice/group from three independent experiments, Mann-Whitney test). (C) HDL infusion reduced mortality and clinical severity of acute GVHD ($n = 19$–39 mice/group from four independent experiments, log-rank test or Kruskal-Wallis and Dunn's post-test on AUC). (D) Six days after transplantation, 3HM was quantified by Endoquant® method in the plasma and in the bile of recipient mice (Plasma: 13 mice/group from four independent experiments; Bile: 9–10 mice/group from four independent experiments). Circulating HDL-cholesterol level was inversely correlated with 3HM concentration in the plasma ($n = 26$ pairs, Spearman $r = -0.6897$, $p < .0001$) and in the bile (data not shown, $n = 19$ pairs, Spearman $r = -0.7912$, $p < .0001$). The activity of circulating LPS was measured by LAL assay in the plasma of allorecipient mice treated or not with HDL infusions ($n = 7$–9 mice/group from three independent experiments). (E) The circulating IL-6 concentration was assessed in the plasma of allo-recipient mice at day+6 after transplantation by ELISA ($n = 10$ mice/group from two independent experiments, unpaired t-test with Welch's correction) (*$p < .05$, ****$p < .0001$)

with a decreased plasma capacity to neutralize LPS activity due to a collapse of circulating HDL concentration. The causes of this decline remain to be determined. We hypothesized that an intensified HDL consumption for the elimination of increased concentrations of LPS and other pro-inflammatory lipids and/or a defect in HDL

synthesis caused by inflammation[36-38] could lead to the low levels of circulating HDL. The relevance of HDL metabolism during aGVHD is confirmed in HDL-deficient *Apoa1*[tm1Unc] recipients, with greater aGVHD severity and mortality. While LPS elimination by HDL particles does not seem to play a major role, an increased APC activation
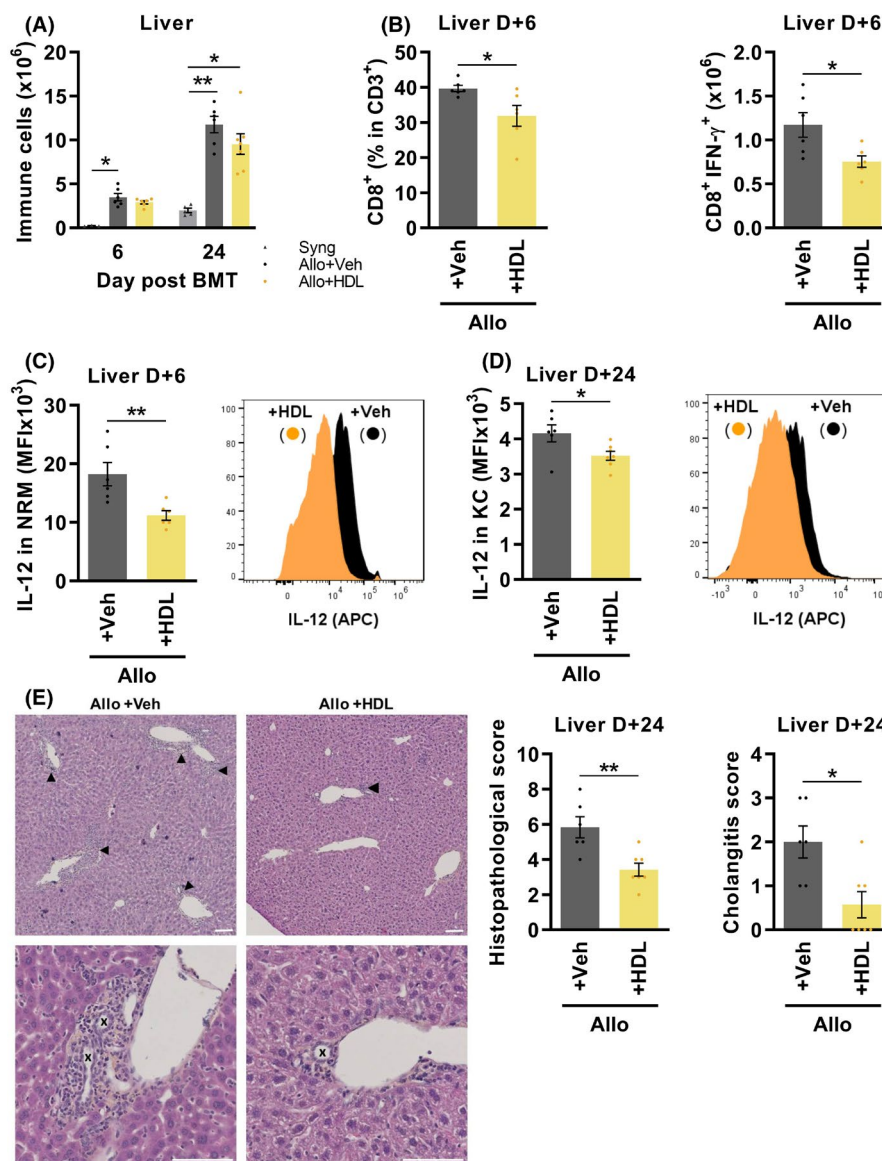
FIGURE 6 HDL infusion limits hepatic GVHD by lowering IFN-γ production by CD8⁺ T cells and pro-inflammatory cytokine production by macrophages in the liver. (A) The number of immune cells was quantified in the mouse liver at D+6 and D+24 after BMT ($n = 3$–7 mice/ group, Kruskal-Wallis and Dunn's post-test). (B) T cell polarization (appreciated by percent of CD8⁺ T cells and absolute number of IFN-γ⁺ CD8⁺ T cells) was analyzed by flow cytometry in the liver of allografted mice on day+6 after BMT ($n = 6$ mice/group, Mann-Whitney test). (C, D) Cytokine secretion of Küpffer cells (KC) and non-resident macrophages (NRM) was quantified by intracellular staining. At day+6 after transplantation, IL-12 secretion by NRM is lowered for the HDL-treated mice whereas IL-12 secretion by Küpffer cells is lowered at day+24 after transplantation ($n = 6$–7 mice/group, Mann-Whitney test). (E) Liver sections harvested 24 days after transplantation were formalin-fixed and paraffin-embedded prior to HE staining and analyzed for lobular hepatitis, portal vein endothelitis, portal inflammation and cholangitis. Arrowheads in upper panels (original magnification ×125) indicate portal inflammation. Cholangitis was quantified as the number of portal tracts displaying bile duct inflammation. Crosses in lower panels (original magnification ×500) indicate bile ducts. The scale bars are equivalent to 100 μm ($n = 5$–7 mice/group, Mann-Whitney test) (*$p < .05$, **$p < .01$)

and Th1/Tc1 polarization in the liver of *Apoa1^tm1Unc* recipients were observed. These data suggest that other mechanisms may explain the worsened GVHD severity because of the lack of HDL or ApoA-I synthesis. Indeed, ApoA-I or HDL have been shown to modulate innate and adaptive immune responses in other pathological situations. These effects on immune cells differ depending on the considered disease.[39-41] In line with our observations, upregulation of Th1 responses and DC maturation have been previously reported

in *Apoa1^tm1Unc* mice in an antigen-induced arthritis model.[39] This suggests that ApoA-I and/or HDL may exert direct immunomodulatory effect, and this may modulate aGVHD. Nevertheless, these observations in *Apoa1^tm1Unc* mice together with the drop of circulating HDL shaped a therapeutic strategy aiming to restore circulating HDL levels. Repeated infusions of HDL isolated from healthy donors limited mortality and, notably, mitigated hepatic aGVHD. HDL infusion reduced pathogenic IFN-γ-secreting CD8⁺ T cell infiltration in

the liver, decreased pro-inflammatory cytokine production by hepatic macrophages and limited histologic lesions, particularly the bile duct inflammation (cholangitis). While a significant decrease in circulating IL-6 is detected after HDL infusion, no effect is observed on IL-17-secreting T cells. These data are in line with a recent study on atherosclerosis showing that while ApoA-I administration reduces significantly plasma IL-6 levels, no change in IL-17^{+} T cells is found.[41] Additional experiments are required to determine the specific role of HDL, ApoA-I and of their receptor, scavenger receptor BI,[42] in our observations.

Some differences exist between mouse and human lipoprotein metabolism and should be considered. As there is a total absence of cholesteryl ester transfer protein (CETP) expression in mice, the major lipoproteins for the transport of cholesterol are HDL.[25] A part of this transport is supported by low-density lipoproteins (LDLs) in humans. This difference could also influence RLT, as LPS is mainly taken up by LDL in patient with systemic inflammatory response syndrome.[43] However, the binding affinity of LPS is superior for HDL than for LDL in healthy donor blood samples.[44] If the LPS activity seems to be also neutralized by LDL, this binding tends to lower the speed of lipoprotein elimination.[45,46] In light of these facts, we decided to keep our study focused on HDL properties.

Hence, HDL infusion appears as an effective aGVHD prophylaxis in our experimental models. The decrease in systemic LPS concentration and activity appears insufficient to explain the significant effect of HDL administration (see above). Several bioactive lipids that may influence aGVHD are transported by HDL and eliminated by the liver. This is the case of other bacterial lipids (e.g., gram-positive bacterial lipoteichoic acid[47]). Pro-inflammatory host-derived oxidized lipids are also neutralized by HDL.[48] Furthermore, as previously mentioned, HDL exert other anti-inflammatory properties by modulating innate and adaptive immune responses.[39,49,50] HDL can interfere with macrophage TLR expression and signaling by downregulating TLR-induced pro-inflammatory cytokines *via* the transcription factor ATF3.[50] HDL can modulate APC functions by stimulating cholesterol efflux; given that cholesterol accumulation in APC increases antigen presentation capacity, inflammasome activation and pro-inflammatory cytokine production.[51,52] Furthermore, HDLs are the main carriers of sphingosine-1-phosphate, a bioactive lipid which plays a regulatory role in cytokine secretion, endothelial barrier function and immune cell migration.[53-55] The exact mechanisms involved in the beneficial effects of HDL administration need to be clarified, but we already demonstrate that the liver may play a central role. Mitigation of liver inflammation and aGVHD hepatic damages by HDL infusion could explain, at least partly, the protective effect of HDL. Notably, HDL-treated mice experienced a less severe cholangitis. Bile duct inflammation was described as a primary mechanism in human and experimental aGVHD[56,57] and could be caused by sepsis or by aGVHD,[58] which are both associated with a higher incidence of non-relapse mortality in allografted patients.[59] The liver appears as a major contributor in the regulation of LPS and lipoprotein metabolisms during aGVHD. Inhibition of reverse lipid transport by local inflammation and danger signal abundance[36] could be limited by HDL infusions. Thus, our results reveal the therapeutic interest of HDL administration in the prevention of aGVHD.

Concerning the clinical relevance of our study, no data exist on circulating HDL levels and aGVHD occurrence after alloHCT. HDL is critical in cholesterol metabolism and modulation of this metabolism has been shown to prevent aGVHD in mice. Indeed, Zeiser et al. demonstrated the prevention of aGVHD by statins that inhibit de novo cholesterol synthesis.[60] A clinical study involving 113 patients reported that hypercholesterolemia in both recipient and donor at time of transplantation is associated with increased aGvHD. However, no significant association was found between HDL or LDL and the incidence of aGVHD.[61] This suggests that HDL together with total and free LPS levels should be monitored in allografted patients to better transpose our data in clinical setting.

DATA AVAILABILITY STATEMENT

Data are available upon request to the corresponding author.

ORCID

Cécile Chagué https://orcid.org/0000-0002-7249-6047
Thomas Gautier https://orcid.org/0000-0003-1119-7382
Jean-Paul Pais de Barros https://orcid.org/0000-0002-5124-2283
Valérie Deckert https://orcid.org/0000-0001-5728-1710
Laurent Lagrost https://orcid.org/0000-0002-8878-6081
Etienne Daguindau https://orcid.org/0000-0001-7375-5656
Philippe Saas https://orcid.org/0000-0002-8857-9939

REFERENCES

1. Hill GR, Crawford JM, Cooke KR, Brinson YS, Pan L, Ferrara JL. Total body irradiation and acute graft-versus-host disease: the role of gastrointestinal damage and inflammatory cytokines. *Blood*. 1997;90(8):3204-3213.
2. Bruce DW, Stefanski HE, Vincent BG, et al. Type 2 innate lymphoid cells treat and prevent acute gastrointestinal graft-versus-host disease. *J Clin Invest*. 2017;127(5):1813–1825.
3. Ferrara JLM, Smith CM, Sheets J, Reddy P, Serody JS. Altered homeostatic regulation of innate and adaptive immunity in

lower gastrointestinal tract GVHD pathogenesis. *J Clin Invest*. 2017;127(7):2441-2451.

4. Troeger H, Hering NA, Bojarski C, et al. Epithelial barrier dysfunction as permissive pathomechanism in human intestinal graft-versus-host disease. *Bone Marrow Transplant*. 2018;53(8):1083-1086.

5. Eriguchi Y, Takashima S, Oka H, et al. Graft-versus-host disease disrupts intestinal microbial ecology by inhibiting Paneth cell production of α-defensins. *Blood*. 2012;120(1):223-231.

6. Peled JU, Gomes ALC, Devlin SM, et al. Microbiota as predictor of mortality in allogeneic hematopoietic-cell transplantation. *N Engl J Med*. 2020;382(9):822-834.

7. Mathewson ND, Jenq R, Mathew AV, et al. Gut microbiome-derived metabolites modulate intestinal epithelial cell damage and mitigate graft-versus-host disease. *Nat Immunol*. 2016;17(5):505-513.

8. Swimm A, Giver CR, DeFilipp Z, et al. Indoles derived from intestinal microbiota act via type I interferon signaling to limit graft-versus-host disease. *Blood*. 2018;132(23):2506-2519.

9. Michonneau D, Latis E, Curis E, et al. Metabolomics analysis of human acute graft-versus-host disease reveals changes in host and microbiota-derived metabolites. *Nat Commun*. 2019;10(1):5695.

10. Price KS, Nestel FP, Lapp WS. Progressive accumulation of bacterial lipopolysaccharide in vivo during murine acute graft-versus-host disease. *Scand J Immunol*. 1997;45(3):294-300.

11. Cooke KR, Hill GR, Crawford JM, et al. Tumor necrosis factor- alpha production to lipopolysaccharide stimulation by donor cells predicts the severity of experimental acute graft-versus-host disease. *J Clin Invest*. 1998;102(10):1882-1891.

12. Zhao YI, Liu Q, Yang LI, et al. TLR4 inactivation protects from graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Cell Mol Immunol*. 2013;10(2):165-175.

13. Cooke KR, Gerbitz A, Crawford JM, et al. LPS antagonism reduces graft-versus-host disease and preserves graft-versus-leukemia activity after experimental bone marrow transplantation. *J Clin Invest*. 2001;107(12):1581-1589.

14. Daguindau E, Gautier T, Chagué C, et al. Is it time to reconsider the lipopolysaccharide paradigm in acute graft-versus-host disease? *Front Immunol*. 2017;8:952.

15. Gautier T, Lagrost L. Plasma PLTP (phospholipid-transfer protein): an emerging role in « reverse lipopolysaccharide transport » and innate immunity. *Biochem Soc Trans*. 2011;39(4):984-988.

16. Azzam KM, Fessler MB. Crosstalk between reverse cholesterol transport and innate immunity. *Trends Endocrinol Metab*. 2012;23(4):169-178.

17. Levels JHM, Marquart JA, Abraham PR, et al. Lipopolysaccharide is transferred from high-density to low-density lipoproteins by lipopolysaccharide-binding protein and phospholipid transfer protein. *Infect Immun*. 2005;73(4):2321-2326.

18. Kitchens RL, Thompson PA, Viriyakosol S, O'Keefe GE, Munford RS. Plasma CD14 decreases monocyte responses to LPS by transferring cell-bound LPS to plasma lipoproteins. *J Clin Invest*. 2001;108(3):485-493.

19. Deng M, Scott MJ, Loughran P, et al. Lipopolysaccharide clearance, bacterial clearance, and systemic inflammatory responses are regulated by cell type-specific functions of TLR4 during sepsis. *J Immunol*. 2013;190(10):5152-5160.

20. Yao Z, Mates JM, Cheplowitz AM, et al. Blood-borne lipopolysaccharide is rapidly eliminated by liver sinusoidal endothelial cells via high-density lipoprotein. *J Immunol*. 2016;197(6):2390-2399.

21. Shao B, Lu M, Katz SC, et al. A host lipase detoxifies bacterial lipopolysaccharides in the liver and spleen. *J Biol Chem*. 2007;282(18):13726-13735.

22. Satoh M, Ando S, Shinoda T, Yamazaki M. Clearance of bacterial lipopolysaccharides and lipid A by the liver and the role of arginino-succinate synthase. *Innate Immun*. 2008;14(1):51-60.

23. Pais de Barros J-P, Gautier T, Sali W, et al. Quantitative lipopolysaccharide analysis using HPLC/MS/MS and its combination with the limulus amebocyte lysate assay. *J Lipid Res*. 2015;56(7):1363-1369.

24. Deckert V, Lemaire S, Ripoll P-J, et al. Recombinant human plasma phospholipid transfer protein (PLTP) to prevent bacterial growth and to treat sepsis. *Sci Rep*. 2017;7(1):3053.

25. Camus MC, Chapman MJ, Forgez P, Laplaud PM. Distribution and characterization of the serum lipoproteins and apoproteins in the mouse, Mus Musculus. *J Lipid Res*. 1983;24(9):1210-1228.

26. Ferrara JLM, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet*. 2009;373(9674):1550-1561.

27. Zannis VI, Fotakis P, Koukos G, et al. HDL biogenesis, remodeling, and catabolism. *Handb Exp Pharmacol*. 2015;224:53-111.

28. Kim SY, Jeong J-M, Kim SJ, et al. Pro-inflammatory hepatic macrophages generate ROS through NADPH oxidase 2 via endocytosis of monomeric TLR4-MD2 complex. *Nat Commun*. 2017;8(1):2247.

29. Blom KG, Qazi MR, Matos JBN, Nelson BD, DePierre JW, Abedi-Valugerdi M. Isolation of murine intrahepatic immune cells employing a modified procedure for mechanical disruption and functional characterization of the B, T and natural killer T cells obtained. *Clin Exp Immunol*. 2009;155(2):320-329.

30. Zhang Y, Shlomchik WD, Joe G, et al. APCs in the liver and spleen recruit activated allogeneic CD8 $^+$ T cells to elicit hepatic graft-versus-host disease. *J Immunol*. 2002;169(12):7111-7118.

31. Zeiser R, Blazar BR. Acute graft-versus-host disease - biologic process, prevention, and therapy. *N Engl J Med*. 2017;377(22):2167-2179.

32. MacMillan ML, Robin M, Harris AC, et al. A refined risk score for acute graft-versus-host disease that predicts response to initial therapy, survival, and transplant-related mortality. *Biol Blood Marrow Transplant*. 2015;21(4):761-767.

33. Zeiser R, von Bubnoff N, Butler J, et al. Ruxolitinib for glucocorticoid-refractory acute graft-versus-host disease. *N Engl J Med*. 2020;382(19):1800-1810.

34. DeFilipp Z, Peled JU, Li S, et al. Third-party fecal microbiota transplantation following allo-HCT reconstitutes microbiome diversity. *Blood Adv*. 2018;2(7):745-753.

35. Battipaglia G, Malard F, Rubio MT, et al. Fecal microbiota transplantation before or after allogeneic hematopoietic transplantation in patients with hematologic malignancies carrying multidrug-resistance bacteria. *Haematologica*. 2019;104(8):1682-1688.

36. McGillicuddy FC, de la Llera Moya M, Hinkle CC, et al. Inflammation impairs reverse cholesterol transport in vivo. *Circulation*. 2009;119(8):1135-1145.

37. Malik P, Berisha SZ, Santore J, Agatisa-Boyle C, Brubaker G, Smith JD. Zymosan-mediated inflammation impairs in vivo reverse cholesterol transport. *J Lipid Res*. 2011;52(5):951-957.

38. Jahangiri A, de Beer MC, Noffsinger V, et al. HDL remodeling during the acute phase response. *Arterioscler Thromb Vasc Biol*. 2009;29(2):261-267.

39. Tiniakou I, Drakos E, Sinatkas V, et al. High-density lipoprotein attenuates Th1 and Th17 autoimmune responses by modulating dendritic cell maturation and function. *J Immunol*. 2015;194(10):4676-4687.

40. Black LL, Srivastava R, Schoeb TR, Moore RD, Barnes S, Kabarowski JH. Cholesterol-independent suppression of lymphocyte activation, autoimmunity, and glomerulonephritis by apolipoprotein A-I in normocholesterolemic lupus-prone mice. *J Immunol*. 2015;195(10):4685-4698.

41. Gaddis DE, Padgett LE, Wu R, et al. Apolipoprotein AI prevents regulatory to follicular helper T cell switching during atherosclerosis. *Nat Commun*. 2018;9(1):1095.

42. Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH, Krieger M. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science*. 1996;271(5248):518-520.

43. Levels JHM, Lemaire LCJM, van den Ende AE, van Deventer SJH, van Lanschot JJB. Lipid composition and lipopolysaccharide binding capacity of lipoproteins in plasma and lymph of patients with

systemic inflammatory response syndrome and multiple organ failure. *Crit Care Med.* 2003;31(6):1647-1653.

44. Levels JH, Abraham PR, van den Ende A, van Deventer SJ. Distribution and kinetics of lipoprotein-bound endotoxin. *Infect Immun.* 2001;69(5):2821-2828.

45. Weinstock C, Ullrich H, Hohe R, et al. Low density lipoproteins inhibit endotoxin activation of monocytes. *Arterioscler Thromb J Vasc Biol.* 1992;12(3):341-347.

46. Schvartz YS, Polyakov LM, Dushkin MI, Pivovarova EN. Modification and clearance of low density lipoproteins during the formation of endotoxin-lipoprotein complexes. *Bull Exp Biol Med.* 2008;145(4):430-432.

47. Levels JHM, Abraham PR, van Barreveld EP, Meijers JCM, van Deventer SJH. Distribution and kinetics of lipoprotein-bound lipoteichoic acid. *Infect Immun.* 2003;71(6):3280-3284.

48. Galbois A, Thabut D, Tazi KA, et al. Ex vivo effects of high-density lipoprotein exposure on the lipopolysaccharide-induced inflammatory response in patients with severe cirrhosis. *Hepatol.* 2009;49(1):175-184.

49. Catapano AL, Pirillo A, Bonacina F, Norata GD. HDL in innate and adaptive immunity. *Cardiovasc Res.* 2014;103(3):372-383.

50. De Nardo D, Labzin LI, Kono H, et al. High-density lipoprotein mediates anti-inflammatory reprogramming of macrophages via the transcriptional regulator ATF3. *Nat Immunol.* 2014;15(2):152-160.

51. Ito A, Hong C, Oka K, et al. Cholesterol accumulation in CD11c+ immune cells is a causal and targetable factor in autoimmune disease. *Immunity.* 2016;45(6):1311-1326.

52. Westerterp M, Gautier EL, Ganda A, et al. Cholesterol accumulation in dendritic cells links the inflammasome to acquired immunity. *Cell Metab.* 2017;25(6):1294-1304.e6.

53. Teijaro J, Walsh K, Cahalan S, et al. Endothelial cells are central orchestrators of cytokine amplification during influenza virus infection. *Cell.* 2011;146(6):980-991.

54. Garcia JGN, Liu F, Verin AD, et al. Sphingosine 1-phosphate promotes endothelial cell barrier integrity by Edg-dependent cytoskeletal rearrangement. *J Clin Invest.* 2001;108(5):689-701.

55. Mandala S, Hajdu R, Bergstrom J, et al. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science.* 2002;296(5566):346-349.

56. Shulman HM, Sharma P, Amos D, Fenster LF, McDonald GB. A coded histologic study of hepatic graft-versus-host disease after human bone marrow transplantation. *Hepatology.* 1988;8(3):463-470.

57. Vierling JM, Hreha G, Wang H, Braun M. The role of biliary epithelial cells in the immunopathogenesis of non-suppurative destructive cholangitis in murine hepatic graft-versus-host disease. *Trans Am Clin Climatol Assoc.* 2011;122:326-335.

58. Kusumi E, Kami M, Kanda Y, et al. Hepatic injury following reduced intensity unrelated cord blood transplantation for adult patients with hematological diseases. *Biol Blood Marrow Transplant.* 2006;12(12):1302-1309.

59. Hogan WJ, Maris M, Storer B, et al. Hepatic injury after nonmyeloablative conditioning followed by allogeneic hematopoietic cell transplantation: a study of 193 patients. *Blood.* 2004;103(1):78-84.

60. Zeiser R, Youssef S, Baker J, Kambham N, Steinman L, Negrin RS. Preemptive HMG-CoA reductase inhibition provides graft-versus-host disease protection by Th-2 polarization while sparing graft-versus-leukemia activity. *Blood.* 2007;110(13):4588-4598.

61. Rivera-Franco MM, León-Rodríguez E, Lastra-German IK, Mendoza-Farias AA. Association of recipient and donor hypercholesterolemia prior allogeneic stem cell transplantation and graft-versus-host disease. *Leuk Res.* 2018;72:74-78.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.