Deficiency of hepatic lipase (HL) may play a role in the lipoprotein abnormalities in chronic inflammatory states which are characterized by reticuloendothelial-system activation and cytokine release. HL triacylglycerol hydrolase activity was measured in hepatic perfusates of livers from autoimmune MRL/lpr mice, which spontaneously develop a condition closely resembling human lupus erythematosus and exhibit spontaneous Kupffer-cell activation after 8 weeks of age, as well as from normal mice treated with *Corynebacterium parvum* or polynosinic-polycytidylic acid complex [poly(I-C)] to induce Kupffer-cell activation. HL activity in MRL/lpr mice older than 8 weeks was 29.5% (P = 0.002) of that in age-matched control MRL/++ mice. Treatment of normal mice with *C. parvum* or poly(I-C) resulted in HL activities 18.6% (P = 0.004) and 13.1% (P = 0.007) respectively of untreated controls. Northern-blot hybridization of liver poly(A)+ RNA showed no differences in HL mRNA abundance in MRL/++ mice compared with the MRL/lpr autoimmune strain after 8 weeks of age, or in normal control mice compared with those treated with *C. parvum*, indicating attenuation of HL activity at the translational or post-translational level. Deficiency of this enzyme may represent one of the mechanisms contributing to the dyslipoproteinaemia of autoimmune disease and chronic infection.

**INTRODUCTION**

A complication of chronic illness caused by infection or autoimmune disease is dyslipoproteinaemia, which may result in premature atherosclerosis [1–5]. Several factors may contribute to the development of these abnormalities in lipid metabolism, which may predispose to premature atherosclerosis [6]. Deficiency of lipoprotein lipase (LPL) and/or hepatic lipase (HL) may play a role in the development of lipoprotein abnormalities in chronic inflammatory states characterized by reticuloendothelial-system activation and cytokine release. LPL is bound to vascular endothelium and is responsible for the hydrolysis of triacylglycerol-rich lipoproteins, including chylomicrons and very-low-density lipoproteins (VLDL) [7]. HL is synthesized by hepatocytes and is present on hepatic sinusoidal endothelial cells [7]. Both enzymes are attached to the endothelium by glycosaminoglycan receptors and can be displaced by exogenously administered heparin. In addition to converting high-density lipoprotein (HDL) into HDL3, HL also has a role in the hydrolysis of lipids in VLDL and intermediate-density lipoproteins (IDL) [8,9].

Decreased LPL and HL activities have been reported in patients with acute bacterial and viral infections [10], and decreased post-heparin plasma lipolytic activity has been reported in an untreated child with hypertriglyceridaemia and active systemic lupus erythematosus [4]. The mechanism of the decrease in activity of these lipases in these various inflammatory conditions may be related, in part, to circulating cytokines, particularly tumour necrosis factor-α (TNF-α). TNF-α, formerly named cachectin, is released by activated reticuloendothelial cells, including hepatic Kupffer cells, and has multiple inflammatory and immunoregulatory properties [11]. It has been shown to decrease LPL activity in cultured human adipocytes at the pretranslational level [12]. Administration of TNF-α to rats, mice and guinea pigs results in a decrease in LPL activity in adipose tissue [13]. In addition, cachectic tumour-bearing mice with marked hypertriglyceridaemia were found to have decreased HL activity [14], suggesting that TNF-α or other inflammatory mediators also may inhibit HL.

Spontaneous TNF-α production by Kupffer cells has been reported in MRL/MpJ-lpr/lpr (MRL/lpr) mice [15], an autoimmune strain which spontaneously develops by 3 months of age a multiorgan disease with histopathological and serological similarities to human systemic lupus erythematosus [16]. Secretion of TNF-α is probably secondary to endogenous Kupffer-cell activation [17]. *In vivo*, reticuloendothelial-system activation in non-autoimmune mice by the anaerobic bacterium *Corynebacterium parvum* and synthetic polynosinic-polycytidylic acid complex [poly(I-C)] also results in TNF-α production by Kupffer cells [18]. In the present study, HL activity, recovered by perfusion of the liver in *situ* with heparin, as well as HL mRNA abundance, were examined in these murine models of Kupffer-cell activation.

**MATERIALS AND METHODS**

**Mice**

C3H/HeN, BALB/c, DBA/2J, MRL/MpJ-lpr/lpr (MRL/lpr) and MRL/MpJ-++/+ (MRL/+++) female mice, 1–6 months old, were obtained from colonies at the Jackson Laboratory, Bar Harbor, ME, U.S.A. MRL/++ mice share over 98% of their genome with MRL/lpr mice, but develop a delayed and milder form of autoimmunity [16]. Therefore, they are a convenient control strain for the MPL/lpr mice. The mice were screened routinely for pathogens and housed in a barrier facility with sterile caging, bedding, water and food.

**Reagents**

*C. parvum* was purchased from Burroughs Wellcome Co., Research Triangle Park, NC, U.S.A., and 1.4 mg was injected
intravenously 3–7 days before mice were killed. Poly(I-C) was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A., and 100 μg was injected intraperitoneally 24 h before killing. Recombinant murine TNF-α (rmTNF-α) was obtained from Genzyme, Cambridge, MA, U.S.A.

Liver perfusions
Mice were anaesthetized with 3 mg of pentobarbital (Nembutal; Abbott Laboratories, Chicago, IL, U.S.A.) administered intraperitoneally. A ventral midline incision was made to expose the peritoneal cavity. The portal vein was cannulated with a 22-gauge angiocatheter (Deseret Medical, Sandy, UT, U.S.A.), and the inferior vena cava (IVC) was cannulated with an 18-gauge angiocatheter (Deseret). The liver was flushed via the portal vein with warmed (37°C) oxygenated Ca²⁺/Mg²⁺-free Krev–Henseleit buffer at pH 7.4, and then perfused at a flow rate of 3 ml/min with the above perfusion buffer plus heparin (150 units/ml) (Sigma). Outflow was collected in serial fractions from the IVC. Only those perfusions in which the entire liver remained blanched and more than 85% of the perfusate volume was recovered from the IVC were used for lipase analysis. After the perfusion, the liver was excised, blotted and weighed.

Lipase assay
Liver perfusate triacylglycerol hydrolase activity was measured as previously described [19]. Each assay volume of 0.250 ml contained 0.200 ml of substrate and buffer and 0.050 ml of liver perfusate. The final assay volume contained 0.075 μCi of glycerol [³H]triol (New England Nuclear, Boston, MA, U.S.A.), 0.75 μmol of unlabelled glycerol triol (Sigma), and 0.15 M (low-salt assay, pH 8.2) or 0.75 M (high-salt assay, pH 8.8) NaCl. Incubations were carried out at 28°C for 60 min and initiated by addition of 0.050 ml of perfusate. Incubations were terminated, and non-esterified fatty acids were extracted and counted for radioactivity by liquid-scintillation spectrometry in a Packard model 2000 scintillation counter. Triacylglycerol hydrolase activity for each liver was calculated as μmol of non-esterified fatty acid produced/h per g of liver tissue.

Hepatic RNA extraction and analysis by Northern blot hybridization
Total hepatic RNA was isolated by a guanidine thiocyanate method [20]. Samples of the RNA were electrophoresed on a 1.2%–agarose gel and checked for integrity of the 28 S and 18 S rRNA by staining with ethidium bromide and observation under u.v. light. Poly(A)-enriched RNA was prepared from the total RNA by oligo(dT)-cellulose affinity chromatography. Electrophoresed samples were transferred to nitrocellulose filters, which were then successively hybridized with a 32P-labelled rat HL cDNA (kindly provided by Dr. Michael Schotz, University of California, Los Angeles, CA, U.S.A.) and murine β-actin cDNA [21]. Prehybridization and hybridization conditions were as described previously [22]. Autoradiographs were developed after exposure to XAR-5 film (Eastman Kodak Co., Rochester, NY, U.S.A.) at room temperature for 3 days, followed by scanning densitometry.

Statistical analysis
Results were statistically evaluated by using Student’s unpaired two-tailed t test, with rejection of the null hypothesis at P < 0.05 (NS, not significant).

RESULTS
Recovery of HL from the IVC
Over 90% of the total HL activity recovered in the IVC was collected in the first 6 min of liver perfusion. No significant variation in total HL activity recovered in the IVC after heparin perfusion of the liver was observed among the non-autoimmune strains and MRL/++ strains of all ages and MRL/lpr mice less than 6 weeks old, when corrected for liver weight. In the present study, we were interested in measuring only HL activity and not LPL; therefore, serum was not added to the low-salt assay. Activities obtained in the parallel high- and low-salt assays were nearly identical.

HL activity of MRL/lpr and MRL/++ mice
HL activity recovered from the IVC after heparin perfusion of the portal vein was measured in female MRL/lpr and MRL/++ mice. Female MRL/lpr mice older than 14 weeks develop serological and histopathological features of multisystem autoimmune disease [16]. However, Kupffer cells of MRL/lpr female mice as young as 8 weeks are activated [17] and spontaneously produce TNF-α in vitro [15]. In contrast, activation and spontaneous production of TNF-α by MRL/++ Kupffer cells have only been observed in mice older than 10 months [15]. Therefore, we focused on 8–12-week-old mice, an age at which Kupffer cells of MRL/lpr mice are activated but there is no evidence of disease. HL activity of MRL/++ mice at all ages examined and of MRL/lpr mice less than 6 weeks old was comparable with the non-autoimmune strains. In contrast, HL activity in untreated MRL/lpr mice older than 8 weeks was 29.5% (P = 0.002) of that of age-matched MRL/++ mice (Figure 1). The difference in HL activity between MRL/lpr and MRL/++ strains was also noted when data were not corrected for differences in liver weight.

Modulation of HL activity in non-autoimmune mice by treatment with C. parvum or poly(I-C)
We next examined the effect of exogenous reticuloendothelial system activation on HL activity. HL activity was assayed in non-autoimmune mice after treatment with C. parvum (1.4 mg intravenously 3–7 days before death) or poly(I-C) (100 μg intravenously 3–7 days before death). We found that treatment of non-autoimmune mice with C. parvum significantly reduced HL activity, whereas HL activity in poly(I-C) treated mice was similar to that of untreated controls.
Intraperitoneally 24 h before death). As shown in Figure 2, treatment of littermates with \textit{C. parvum} and poly(I:C) resulted in HL activity 18.6% (\(P = 0.004\)) and 13.1% (\(P = 0.007\)), respectively, of untreated controls.

**Figure 3** Representative Northern-blot hybridizations of liver HL and \(\beta\)-actin mRNA from MRL/++ (lane 1) and MRL/lpr (lane 2) mice, as well as C3H control (lane 3) and \textit{C. parvum}-treated (lane 4) mice

A 10 \(\mu\)g portion of poly(A)+ RNA was loaded in each lane. Blots were successively hybridized with \(^{32}\)P-labelled rat HL (left panel) and murine \(\beta\)-actin (right panel) cDNAs.

**Figure 4** Effect of rmTNF-\(\alpha\) on the release of HL by liver perfusion

Livers of non-autoimmune control mice (C3H/HeN) were perfused as described in the Materials and methods section with PBS, PBS plus 67 ng/ml rmTNF-\(\alpha\), and PBS plus 150 units/ml heparin. HL activity was measured and calculated as \(\mu\)mol of FFA released/h per g of liver. Activity is expressed in this graph as the percentage of total recovery activity (PBS plus heparin). Each bar represents the mean of duplicate measurements from two animals.

**DISCUSSION**

We have observed decreased hepatic lipase activity in two murine models of reticuloendothelial activation, both endogenous (MRL/lpr mouse strain) and exogenous (M. parvum and poly(I:C) treatment of non-autoimmune strains). To our knowledge, our findings in the MRL/lpr mouse strain represent the first report of an acquired decrease in HL activity in an untreated non-tumour-bearing animal model. Since HL mRNA levels were not significantly changed, the inhibition may be mediated at the
translational or post-translational level. The specific role of Kupffer-cell activation in the modulation of HL activity remains to be elucidated.

TNF-α has been shown to decrease adipose-tissue LPL activity both in vivo in the rat [13,25] and in cultures of murine and human adipocytes [12,26-29]. Such inhibition appears to be secondary to decreased synthesis of LPL, accompanied by a parallel decrease in LPL mRNA abundance. Our results with HL differ from the previous studies of LPL in that no changes in HL mRNA abundance occurred in the face of decreased enzyme activity. We also investigated the possibility that TNF-α might cause the release of HL from its receptors on the hepatic sinusoidal endothelial cell and result in the recovery of decreased activity after heparin perfusion of the liver, but our results indicate that HL release does not occur in response to liver perfusion with TNF-α.

Other possible mechanisms for the observed decrease in HL activity in MRL/lpr and Kupffer-cell-activated non-autoimmune mice include inhibition by other inflammatory mediators released by activated macrophages or in response to TNF-α, hormonal changes and hepatic parenchymal injury. TNF-α induces the release of interleukin-1 from endothelial cells [30]. Zechner et al. [27] demonstrated that TNF-α, but not interleukin-1, downregulated LPL gene expression in mouse 3T3-L1 adipocytes, although interleukin-1 did decrease LPL activity. Also interferon-γ has been shown to decrease heparin-releasable LPL activity from 3T3-L1 cells [28]. A probable insulin-mediated decrease in HL activity has been observed in rats with starvation and diabetes [31,32]. However, in the present study all animals maintained a similar food intake. Although severe liver parenchymal injury can decrease HL activity [19], the autoimmune strain used in the present study has previously been shown to have only a mild triaditis without liver cell necrosis on histological examination of liver tissue [16]. Furthermore, C. parvum and poly(I-C) are not known to be directly hepatotoxic. Finally, we cannot rule out the possibility of the formation of anti-HL autoantibodies in the MRL/lpr mice, as has been described in a patient with hyperlipidaemia and autoimmune disease [33]. However, this mechanism is not likely to explain our results in the non-autoimmune mice.

In conclusion, we report decreased HL activity in MRL/lpr mice older than 8 weeks and in non-autoimmune mice treated with macrophage activators. This decrease in HL activity is not accompanied by a decrease in HL mRNA abundance, as assessed by Northern-blot hybridization. Deficiency of this enzyme may represent one of the mechanisms contributing to the dyslipoproteinaemia of autoimmune disease and chronic infection. Elucidation of the cellular mechanisms by which reticulo-endothelial activation in the models used in this study may be inhibiting HL will be the focus of future investigation.

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