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# Inflammatory changes in peripheral organs in the BACHD murine model of Huntington's disease

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ABSTRACT

Huntington's disease (HD) is a neurodegenerative disease caused by a CAG repeat expansion in the gene encoding the huntingtin protein (HTT). This expansion leads to the formation of mutant huntingtin protein (mHTT) that is expressed in many body tissue cells. The mHTT interacts with several molecular pathways within different cell types, affecting the regulation of the immune system cells. It is still very limited the understanding of the immune changes in peripheral tissues in HD. Herein, we investigated the levels of inflammatory and regulatory cytokines in peripheral organs (i.e. kidney, heart, liver and spleen) of the 12-month-old BACHD model of HD. This robust murine model closely resembles the human disease. We found significant changes in cytokine levels in all organs analyzed. Increased levels of IL-6 were found in the kidney, while levels of IL-6 and IL-12p70 were increased in the heart of BACHD mice in comparison with wild-type (WT) animals. In the liver, we observed enhanced IL-12p70 and TNF-α levels. In the spleen, there was an increase in the levels of IL-4 and a decrease in the levels of IL-5 and IL-6 in BACHD compared to WT. Our findings provide the first evidence that the BACHD model also exhibits immune changes in peripheral organs, opening an avenue for the investigation of the potential role played by peripheral inflammatory response in HD. Further studies are needed to systematically address the mechanisms and pathways underlying immune signaling in peripheral organs in HD.

## 1. Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease characterized by loss of neurons in the striatum and cerebral cortex, leading to progressive motor dysfunction, cognitive decline and behavioral symptoms [1,2]. HD is caused by the expansion of the CAG trinucleotide repeats in the exon 1 of the gene that encodes the huntingtin (HTT), thus generating a mutant form of this protein [3,4].

There is no treatment for this fatal disease. Over the past years, new therapeutic agents have been investigated, especially those with immunomodulatory and/or anti-inflammatory properties [5,6]. Indeed, it has been reported a role for the immune system in HD as the disease-related neuronal death leads to astrocyte and microglia activation with subsequent release of inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β [7–9]. These cytokines, in turn, promote additional activation of microglia, resulting in an inflammatory milieu that contributes to a cycle of inflammation and neuronal damage [10,11].

Although HD is classically conceived as a central nervous system (CNS) disease, emerging clinical and experimental research has reported significant changes in peripheral organs, determining heart failure, testicular atrophy and impaired pancreatic function as evidenced by impaired glucose tolerance [12–16]. The discovery that both normal HTT and its mutated form are expressed in peripheral tissues and organs from humans and other mammals, such as liver, heart, kidney, pancreas, testicles and stomach, challenged the assumption that HD-related symptoms would be restricted to the CNS [4,17].

HD-related peripheral changes seem to occur even before the onset of the cardinal motor symptoms of the disease [4,18,19]. Using murine

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models of HD, one study showed that changes in immune response occur before the onset of major neurological symptoms [20]. This study also showed a differential activation of genes associated with immune pathways in the spleen of R6/1 and zQ175 mice following active immunization with a combination of three non-overlapping HTT Exon1 coded peptides [20].

The hypothesis that HD-related pathological changes could be found in non-neuronal cells had been formulated nearly one decade before the discovery of the HD gene. In this regard, one study evaluated abnormalities in peripheral blood lymphocytes of patients with HD [21]. Using flow cytometry combined with the fluorescent membrane probe 8-anilino-l-naphthalene sulfonate (ANS), a membrane marker, Gollin et al. [21] observed increase in ANS fluorescence intensity in lymphocytes harvested from patients with HD in comparison with controls, suggesting. These results suggested that membrane changes can occur in peripheral cells such as leukocytes and that selective changes in these cells of patients with HD may reflect altered immune activity reported in this disease [21]. This hypothesis has been confirmed by further studies showing that mHTT can be found in different cell types, including lymphocytes (reviewed in [22]). Western blotting analysis also revealed a higher ratio of mHTT/HTT specifically in platelets, a finding that has been seen at all stages of HD [23]. Noteworthy, HTT mRNA expression is higher in immune cells compared to other tissues and organs under physiological conditions (Genomics Institute of the Novartis Research Foundation, transcript 202389_s_at; [6]). Since the same promoter controls both the wild-type HTT and mHTT expression, it is reasonable to assume that mHTT is also highly expressed in immune cells, potentially affecting their function [6]. In fact, mHTT levels are higher in monocytes and lymphocytes of patients with a clinical diagnosis of HD than in premanifest HD gene carriers and controls [24].

Regarding the immune system, changes in circulating levels of cytokines have been described in HD. For instance, increased plasma levels of IL-6, TNF-α, IL-10 and IL-4 were found in patients at moderate HD stage [25] or at the premanifest phase [18] in comparison with controls. Interestingly, central and peripheral inflammatory changes in HD seem to be connected as the levels of IL-6 and IL-8 are closely correlated in plasma and cerebrospinal fluid of HD patients [18]. Moreover, peripheral inflammation in HD seems to be associated with HD progression (for a review, see [26]). Despite the great piece of evidence derived from studies investigating immune markers in blood samples, it is not clear whether the inflammatory response in peripheral organs is associated with HD pathological processes.

Murine models of HD have become powerful tools for studying potential molecular and cellular mechanisms underlying HD pathogenesis in both CNS and peripheral organs [27]. The BACHD murine model, unlike other animal models of HD, expresses the full length of human mHTT [28]. It has a sequence inserted into the Bacterial Artificial Chromosome (BAC) and contains 240 kb; 170 kb of mutated human HTT, in addition to 20 kb in 5′ end and 50 kb at the 3′ end, thus flanking the *HTT* genomic sequence [28]. The presence of both behavioral and pathological characteristics of the human disease strengthens this model [28,29]. In contrast with other HD models such as $HDH^{150}$ and R6/2, the polyglutamine sequence CAA/CAG repeat length in BACHD mice seems to remain stable in 97 repeats over generations [28–30]. Accordingly, the BACHD model has greater reliability for the study of long-term phenotypic characteristics [29,30].

Taking advantage of the BACHD murine model of HD, this study was designed to define the inflammatory profile in peripheral organs. Our results provide a fundamental step toward the understanding of the inflammatory response in vital peripheral organs and their implications in the pathogenesis and progression of HD.

## 2. Materials and methods

### 2.1. BACHD mice

All experiments were performed according to the rules established by the local animal care committee (Ethics Committee on Animal Experiments of the Universidade Federal de Minas Gerais - CEUA/UFMG; approved protocol #036/2013). All efforts were made to minimize animal suffering and to reduce the number of animals used. For all experiments, mice from both genotypes (WT and BACHD) were deeply anesthetized with ketamine/xylazine (0.1 mL/20 g).

Male FVB/NJ (wild-type or WT) and FVB/N-Tg (HTT*97Q)IXwy/J (BACHD) transgenic mice were purchased from Jackson Laboratory (Barl Harbor, ME, USA) (JAX stock #008197) and used to establish a colony. Mice were held in a place with controlled temperature (23 °C) in a 12-12 h light-dark cycle. Food and water were provided ad libitum. All animals used in this study were genotyped ten days after birth using multiplex Polymerase Chain Reaction (PCR) (HTT-Forward: CCGCTCA GGTTCTGCTTTTA/HTT-Reverse: GGTCGGTGCAGCGGCTCCTC; Actin-Forward: TGGAATCGTGTGGCATCCATCA/Actin-Reverse: AATGCCTG GGTACATGGGGTA).

Animals were identified by numbers according to their genotype (WT or BACHD). They were separated into mini-isolator cages with a maximum of 4 animals per cage. Our experiments were performed with 12-month-old WT and BACHD animals as previous studies using this model demonstrated clear neurodegenerative signals in the cerebral cortex and motor deficits at this age ([28]; for review see [31]). At this age, BACHD mice also exhibit changes in the heart and other muscles such as sternomastoid and diaphragm [32–34].

### 2.2. Assessment of cytokines in peripheral organs

Peripheral organs (kidney, heart, liver and spleen) of WT and BACHD mice (n = 5 per group) were carefully removed and homogenized in an extraction solution (100 mg of tissue per milliliter) containing 0.4 M NaCl, 0.05% Tween 20, 0.5% BSA, 0.1 mM phenyl methyl sulphonyl fluoride, 0.1 mM benzethonium chloride, 10 mM EDTA, and 20 KIU aprotinin, using Ultra-Turrax. Lysates were centrifuged at 13,000 × *g* for 10 min at 4 °C and supernatants were collected and stored at −70 °C until use. Blood samples were also obtained. The samples were centrifuged at 1500 × *g* for 10 min at 4 °C, the serum was collected and stored at −70 °C until analysis.

Inflammatory marker levels (TNF-α, IFN-γ, IL-4, IL-5, IL-6 and IL-12p70) in peripheral organs were simultaneously measured by the Luminex® technique using the Mouse Inflammatory Biomarkers kit (Merck Millipore, Darmstadt, Germany) according to the procedures supplied by the manufacturer. Samples were acquired in a Luminex xMAP® instrument (MAGPIX®, Merck Millipore, Darmstadt, Germany) using the Exponent® software (LUMINEX, Austin, TX, USA). Data were analyzed by MILLIPLEX® Analyst 5.1 (Merck Millipore, Darmstadt, Germany) and results expressed as picograms per 100 mg of tissue.

Inflammatory marker concentrations in serum were determined by ELISA (R&D Systems, Minneapolis, MN) in accordance to the manufacturer's instructions. Results are expressed as picogram per milliliter of serum. The detection limit of the ELISA assay was 5 pg/mL.

### 2.3. Statistical analysis

Results obtained were presented as mean ± SD. All data were tested for normality by employing the Shapiro-Wilk test. For variables normally distributed, differences were compared with the Student's *t*-test. In case of variables not normally distributed, differences were analyzed with the Mann–Whitney *U* test. The significance level was set at $p < 0.05$. Statistical analyses were performed using Prism 5 software (GraphPad, La Jolla, CA, USA).

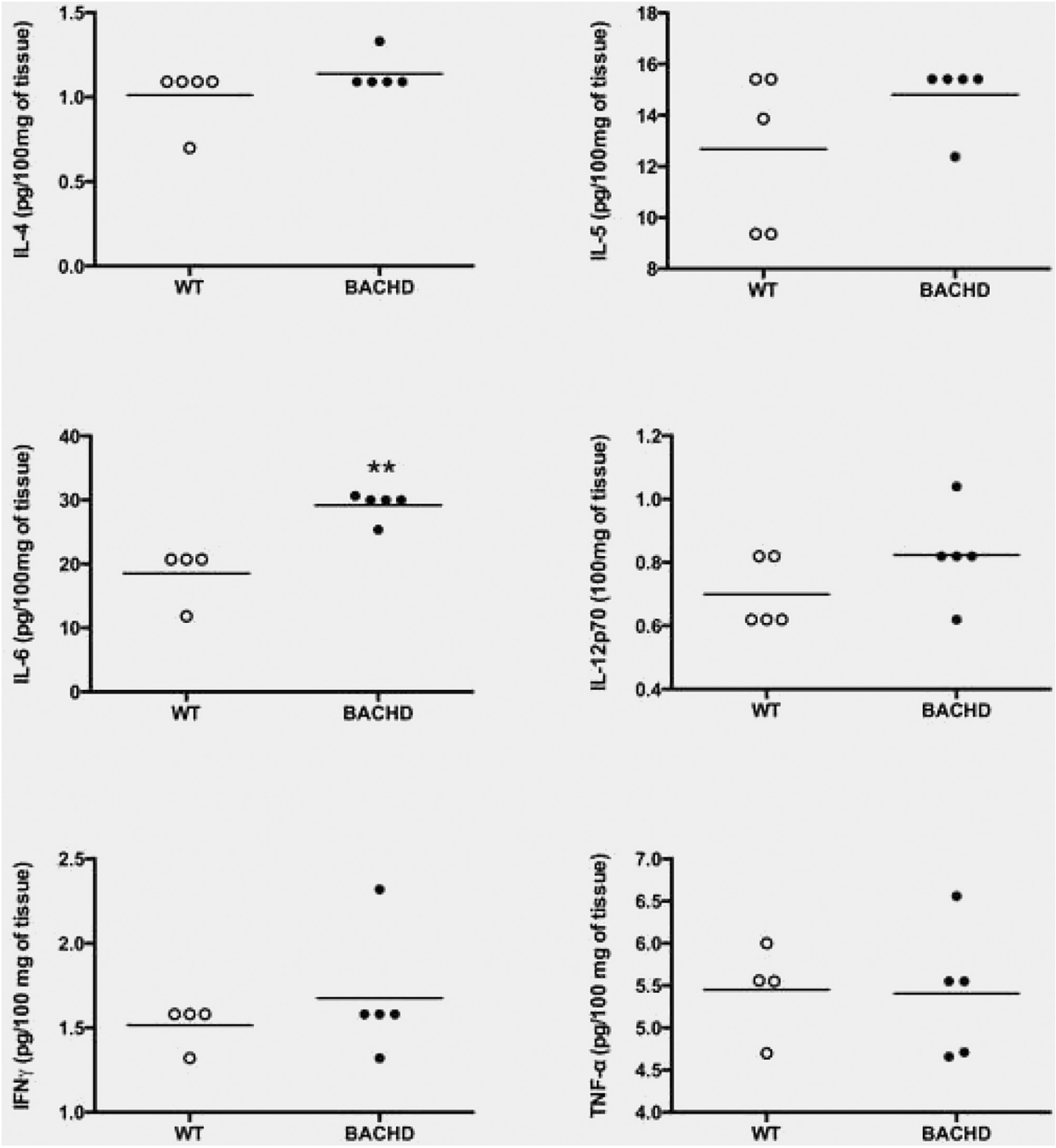


**Fig. 1.** Increased IL-6 cytokine concentration in the kidney of BACHD animals. This figure shows the graphical quantification of cytokines concentration in the WT and BACHD mice kidney. The results are shown as mean ± SD from n = 4 or 5 individual animals per genotype. $^{**}p = 0.001$.

## 3. Results

Over the past years, significant changes in peripheral organs have been reported in clinical and experimental studies of HD [12–16]. Moreover, expressed in many peripheral organs like kidney, heart, liver and spleen, mHTT interacts with several molecular pathways influencing, among others, the immune system [35]. In this context, we measured the concentrations of pro-inflammatory cytokines (IL-6, IL-12p70, IFN-γ, TNF-α,) and anti-inflammatory cytokines (IL-4, IL-5) in the kidney, heart, liver and spleen of BACHD mice.

### *3.1. BACHD mice present increased levels of the cytokine IL-6 in the kidney*

Higher levels of the cytokine IL-6 were found in the kidney of BACHD compared with WT mice (BACHD: 29. 18 ± 2.16 Vs. WT: 18.51 ± 4.43; $^{**}p = 0.001$) (Fig. 1C). No significant differences were observed in the levels of IL-4 ($p = 0.20$), IL-5 ($p = 0.19$), IL-12p70 ($p = 0.17$), TNF-α ($p = 0.92$) and IFN-γ ($p = 0.44$) (Figure. 1).

### *3.2. BACHD mice present increased levels of the cytokines IL-6 and IL-12p70 in the heart*

The heart of BACHD mice presented increased levels of IL-6

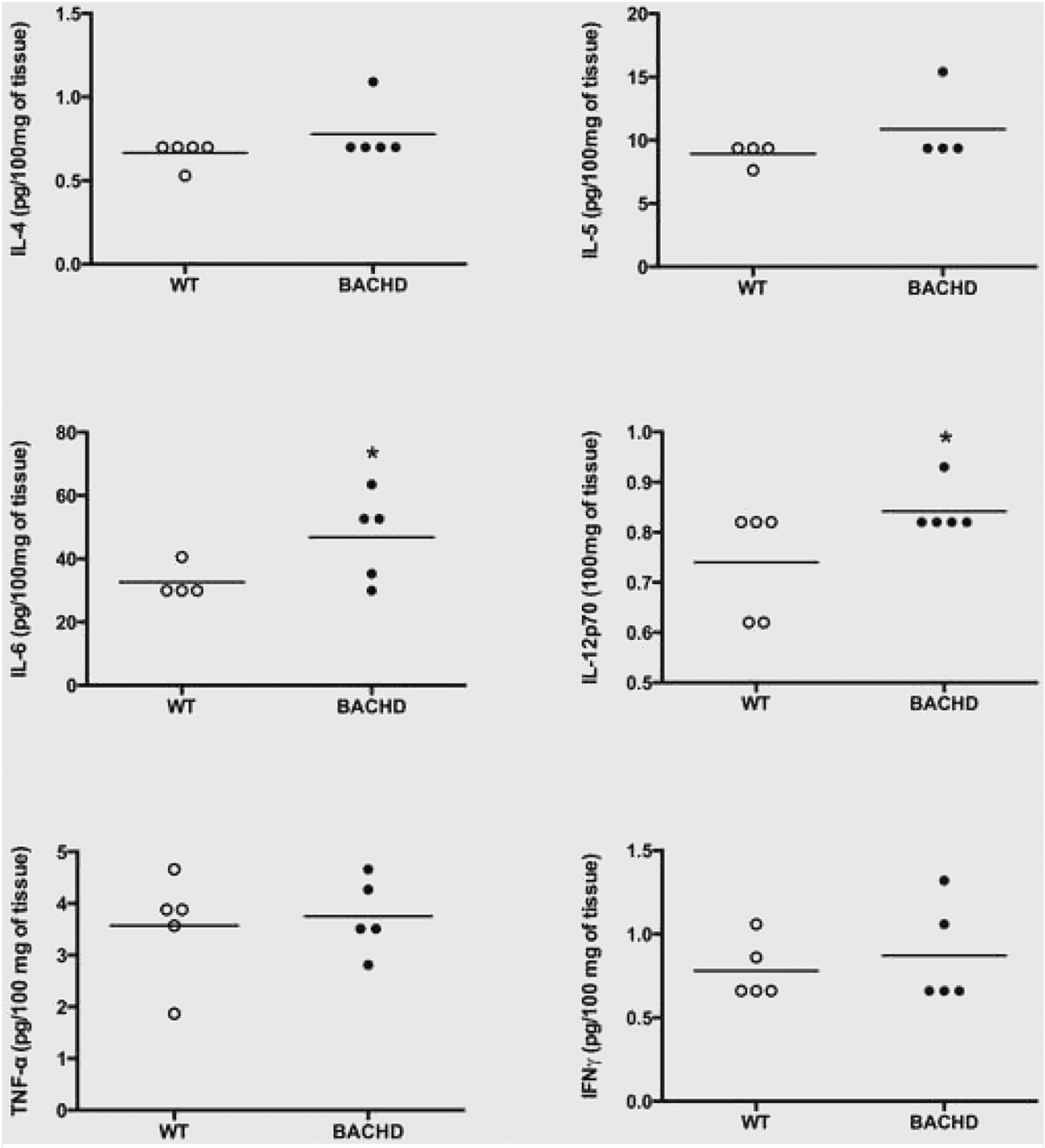


**Fig. 2.** Increase concentration of IL-6 and IL-12p70 cytokines in the heart of BACHD animals. This figure shows the graphical quantification of cytokines concentration in the heart of WT and BACHD animals. The results are shown as mean ± SD from n = 4 or 5 individual animals per genotype. *$p = 0.04$ for IL-6 and IL-12p70.

(BACHD: 46.81 ± 13.82 Vs. WT: 32.62 ± 5.30; *$p = 0.04$) (Fig. 2C) and IL-12p70 (BACHD: 0.84 ± 0.04 vs WT: 0.74 ± 0.10; *$p = 0.04$) (Fig. 2D) compared with WT counterparts. No significant differences were found in the heart levels of the other cytokines: IL-4 ($p = 0.22$), IL-5 ($p = 0.26$), TNF-α ($p = 0.75$), and IFN-γ ($p = 0.28$) (Fig. 2).

### 3.3. BACHD mice show higher hepatic concentration of the inflammatory cytokines IL-12p70 and TNF-α

We observed increased levels of IL-12p70 (BACHD: 1.01 ± 0.12 Vs. WT: 0.72 ± 0.10; **$p = 0.003$) (Fig. 3D), and TNF-α (BACHD: 6.43 ± 1.29 Vs. WT: 4.55 ± 0.72; *$p = 0.03$) (Fig. 3E) in liver samples of BACHD mice in comparison with WT animals. No significant changes were found in the hepatic levels of the cytokines IL-4 ($p = 0.49$), IL-5 ($p = 0.69$), IL-6 ($p = 0.95$) and IFN-γ ($p = 0.96$) (Fig. 3).

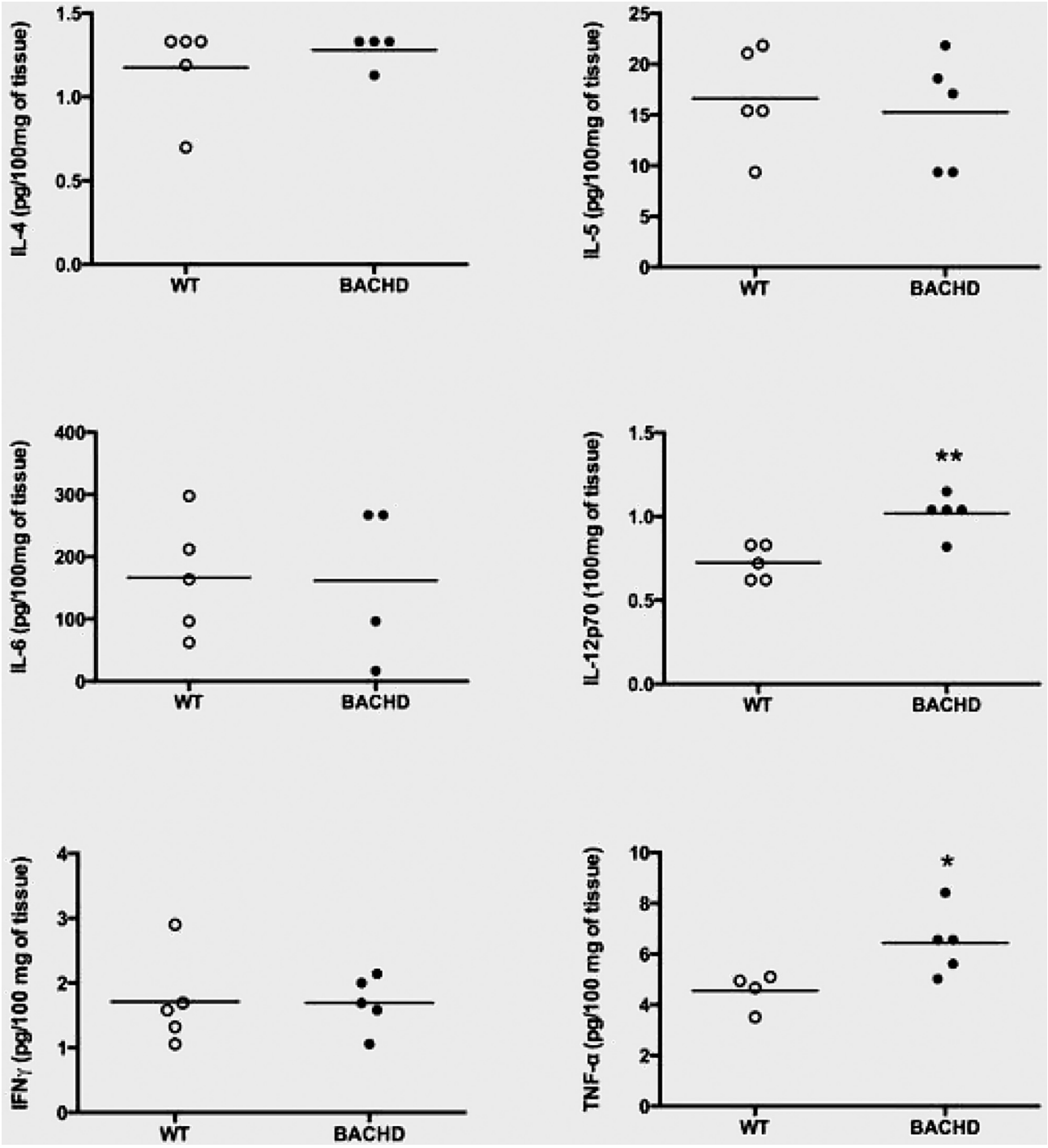


**Fig. 3.** Increased levels of inflammatory cytokines IL-12p70 and TNF-α in the liver of BACHD animals. This figure shows the graphical quantification of cytokines levels in the liver of WT and BACHD animals. The results are shown as mean ± SD from n = 4 or 5 individual animals per genotype. $^{**}p = 0.003$ for IL-12p70 and *0.03 for TNF-α.

### *3.4. BACHD mice exhibit increased levels of the cytokine IL-4 and decreased levels of IL-5 and IL-6 in the spleen*

The levels of the cytokine IL-4 significantly increased in the spleen of BACHD mice, whereas the levels of the cytokine IL-5 and IL-6 decreased when compared with WT animals (IL-4 - BACHD: 3.09 ± 0.34 Vs. WT: 2.04 ± 0.33; $^{**}p = 0.009$), (IL-5-BACHD: 5.87 ± 4.02 Vs. WT: 22.81 ± 1.74; $^{**}p = 0.001$) and (IL-6 - BACHD: 18.61 ± 8.65 Vs. WT: 201.3 ± 183.8; $^{*}p = 0.04$). No significant differences were found in the spleen concentration of the cytokines IL-12p70 ($p = 0.29$), TNF-α ($p = 0.46$) and IFN-γ ($p = 0.75$) (Fig. 4).

### *3.5. BACHD mice present no changes in the serum levels of cytokines*

No significant differences were found in the serum levels of cytokines when comparing BACHD mice with WT animals: IL-4 ($p = 0.87$); IL-5 ($p = 0.75$); IL-6 ($p = 0.85$); IL-12p70 ($p = 0.57$); IFN-γ ($p = 0.86$) and TNF-α ($p = 0.33$) (Fig. 5).

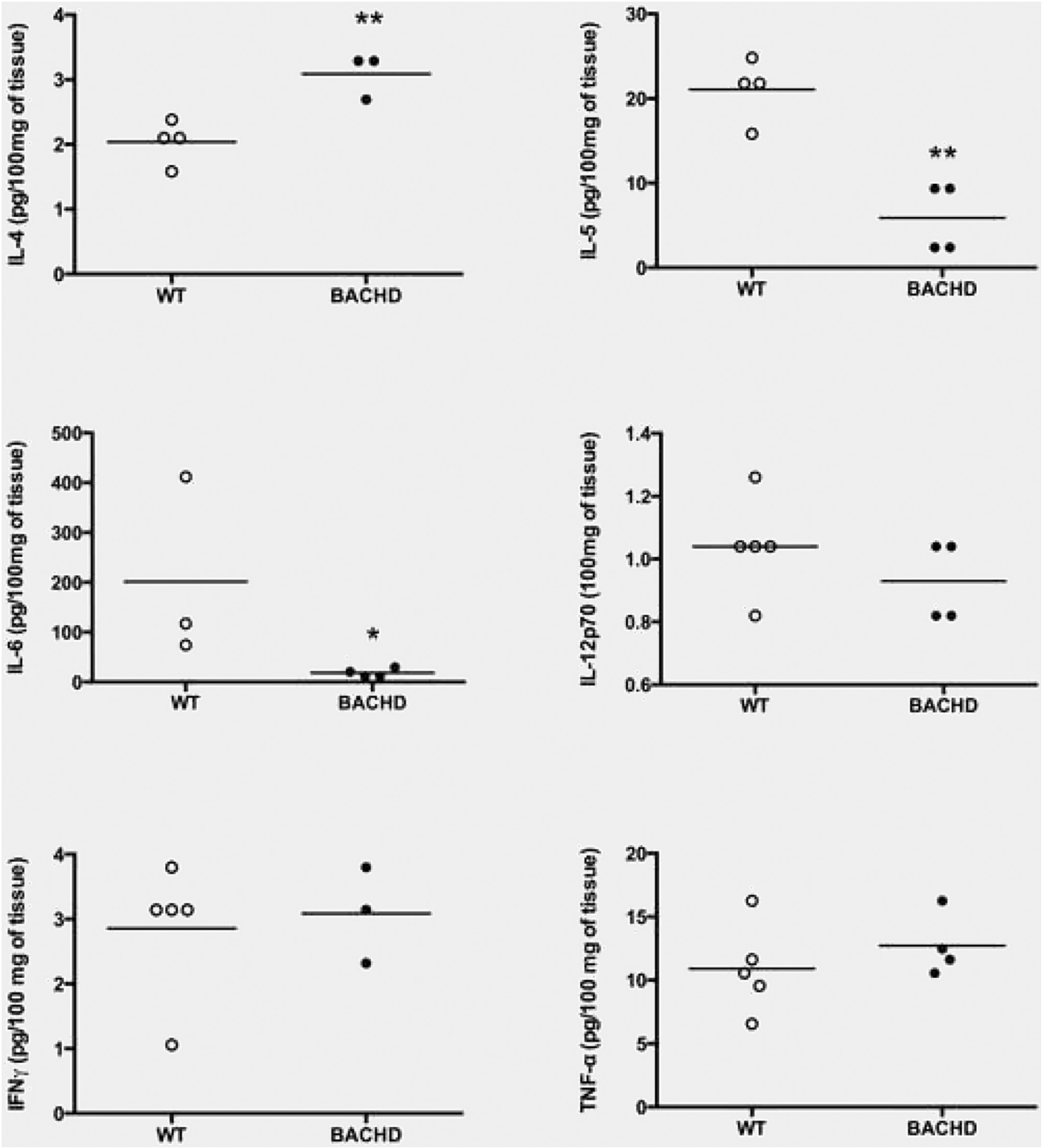


**Fig. 4.** BACHD mice show increased levels of IL-4 and decreased levels of IL-5 and IL-6 in the spleen. This figure shows the graphical quantification of cytokines levels in the heart of WT and BACHD animals. The results are shown as mean ± SD from n = 4 or 5 individual animals per genotype. $^{**}p = 0.009$ for IL-4, $^{**}p = 0.001$ for IL-5 and $^{*}p = 0.04$ for IL-6.

## 4. Discussion

Emerging evidence has proposed HD as a systemic condition rather than an exclusively CNS disease [4,36,37]. In addition to HD-associated neuroinflammation (for review see [22]), it has been reported that patients with HD exhibit peripheral immune changes, including circulating and tissue-based immune cells [38].

To the best of our knowledge, this is the first study to investigate inflammatory changes in peripheral organs in the BACHD mice. We used the BACHD model because it closely recapitulates the disease in humans. Briefly, the current study revealed that the BACHD mice present changes in inflammatory mediators in peripheral organs such as kidney, heart, liver, and spleen, mainly toward a proinflammatory profile.

We found elevated levels of IL-6 in the kidney of BACHD mice. Inflammation in this organ has previously been associated with progressive renal damage, including glomerulonephritis, acute or chronic kidney failure [39]. In addition, chronic kidney disease (CKD) directly influences circulating levels of cytokines and IL-6 is one of the most frequently associated with this pathology [40]. By employing a gene

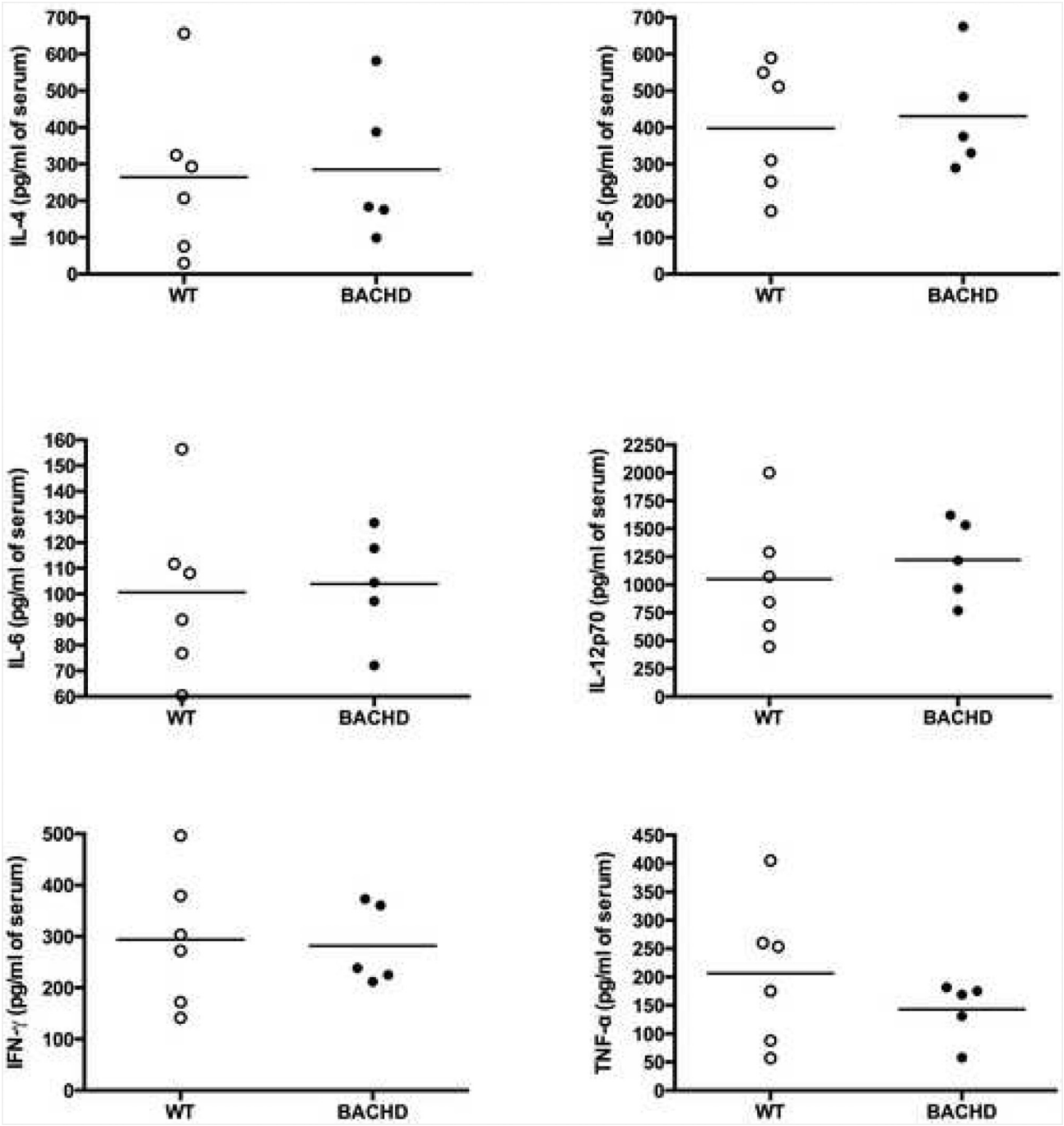


**Fig. 5.** BACHD mice present no changes in the serum levels of cytokines. This figure shows the graphical quantification of cytokines levels in the serum of WT and BACHD animals. The results are shown as mean ± SD from n = 5 or 6 individual animals per genotype.

expression profile analysis, an elegant study showed that patients with CKD have higher expression of IL-6 in monocytes collected from peripheral blood compared with healthy subjects, indicating a role for this cytokine in kidney diseases [41]. Moreover, J774A.1 macrophages and primary mouse peritoneal macrophages stimulated with lipopolysaccharide (LPS) from *E. coli* had higher expression of inflammatory mediators including IL-6 in the presence of indoxyl sulphate, a protein-bound uremic toxin associated with CKD [42]. On the other hand, there is also clinical and preclinical evidence suggesting that IL-6 might induce either renal protection (e.g. diabetic nephropathy) or damage (e.g. glomerulonephritis) depending on the type of the kidney disease (for review see [43]). Further studies are definitely warranted to address the role played by IL-6 in HD-related kidney dysfunction. It is unclear whether this cytokine mediates renal failure/damage or protection in HD, while the impact on renal function of IL-6 increase in the kidney of both humans and animal models of HD [44,45] must be investigated.

As in the kidney, IL-6 levels were also increased in the heart of BACHD mice. It is known that IL-6 signaling in cardiomyocytes exerts a protective effect during the acute response of inflammation. However, when damage persists and IL-6 levels remain chronically elevated, IL-6 can decrease the contractile function of the heart [46–48]. Accordingly, our research group recently identified that the cardiomyocytes of 12-month-old BACHD mice exhibit electromechanical abnormalities with longer action potential, arrhythmic contractions and relaxation disorders [32]. In parallel with these physiological changes, structural abnormalities were described in the mitochondria of cardiomyocytes

derived from BACHD heart ventricles, an indicative of oxidative stress, which was confirmed by imbalance in the activities of superoxide dismutase and glutathione peroxidase [32]. Cardiac dysautonomia has been described in HD patients [49], while mHTT causes cardiac alterations in animal models of HD (R6/2, Drosophila) [14,50].

Although the relationship between inflammation and heart damage has been well described, very few studies had investigated inflammatory mechanisms associated with heart function in mice models of HD. For instance, the cardioprotective effect of B307 treatment – an herbal formulation – was investigated in aging HD R6/2 mice [51]. R6/2 mice had higher expression of TNF-α in the heart, and the expression of this inflammatory marker was associated with cardioprotection after oral administration of B307 [51]. Another study showed that pre-symptomatic BACHD mice (3 months old) exhibited transcriptional changes related to biological processes like apoptosis, gene expression, proliferation and proteolysis/ubiquitination [52]. Corroborating our results, these authors also showed a significant increase in the heart levels of the inflammatory cytokine IL-6, which could contribute to the cardiac changes observed. In addition, a clinical study conducted in a large cohort of early symptomatic HD patient's revealed significant contractile cardiac dysfunction [53]. Altogether, these data suggest that inflammatory processes occur in the heart of HD animal models.

We also showed that the levels of IL-12p70 are increased in heart samples of BACHD mice. Interestingly, IL-12 has been associated with the induction of autoimmune myocarditis in mice [54]. This finding came mainly from the observation that knockout mice for IL-12p40 and IL-12Rβ1 do not develop autoimmune myocarditis [54]. In addition, functional changes such as cardiac contractility and arrhythmias due to conduction system damage are present in autoimmune myocarditis [55,56]. It is tempting to speculate that the changes previously shown by our research group, such as arrhythmic contractions and relaxation disorders in BACHD mice [32], are related to the increase in IL-12p70 levels observed in the heart of BACHD mice.

TNF-α and IL-12p70 levels are increased in the liver of BACHD mice. TNF-α has been implicated in a range of infectious, malignant conditions and inflammation in the liver [57–61]. On the other hand, IL-12p70 has been linked to hepatic steatosis [62]. Since mHTT aggregates have also been found in the liver of animal models (R6/2), liver function defects may contribute to HD pathology [63]. Studies in R6/2 showed that these mice have changes in the urea cycle and dysfunction of hepatic transcriptional factors [64,65]. In humans, the level of alkaline phosphatase is increased in patients with HD in comparison with premanifest HD gene carriers [66]. Liver structure was found altered in biopsies of six patients with HD, and one of the patients had inflammatory cells in the liver [67]. In addition, other studies have shown that HD gene manifest carriers exhibit abnormal blood glucose response after exercise which could be related to changes in hepatic glucose production [12].

There is evidence that the CAG repeats continually expand over time in the liver in murine models of HD such as the $Hdh^{Q111}$. By mapping the genomic DNA of the liver and striatum of $Hdh^{Q111}$ mice, Lee et al. [68] showed that the repetition instability in these two tissues increased over the time [68], indicating that the repetition expansion is a recurring process in HD models [69,70]. Importantly, this expansion might influence liver functioning. The BACHD murine model expresses the complete human Htt gene [28] with a mutant full-length sequence coding for a polyglutamine stretch that is more stable through generations compared to the R6/1 and R6/2 mice models [29,30,71]. Unlikely other HD models such as $HDH^{150}$ and R6/2, the CAA/CAG repeat length in BACHD mice seems to remain stable in 97 replicates over many generations, which increase the reliability of the results in the liver in this model [28].

Interestingly, the spleen was the only organ in which cytokine levels (i.e., IL-5 and IL-6) were decreased in BACHD mice compared to WT animals. On the other hand, we found an increase in IL-4 levels. The reduction in the IL-6 levels in the spleen of BACHD mice was greater than 50% when compared to controls. It is worth mentioning that contrary to the severe weight loss observed in patients with HD, the BACHD mice present a significant increase in body weight [27,28] which might explain, at least in part, the decrease in IL-6 levels in the spleen. A previous study showed that mRNA expression of IL-6 in the spleen of obese animals was decreased by more than 50% [72]. Conversely, Trager et al. [73] showed that spleen myeloid cells from the $HdhQ^{150}$ mouse model have significantly elevated levels of IL-6. The difference among HD models must be taken into account, especially due the weight gain observed in BACHD mice.

BACHD mice develop glucose intolerance and insulin resistance, being considered a pre-diabetes model. Studying metabolic-related abnormalities in BACHD mice, a previous study described very low or undetectable levels of the cytokines IL-6, TNF-α, IL-1β and IFN-γ in serum samples of these animals [74]. Herein, we found no significant differences in the cytokine levels in the serum of BACHD mice in comparison with WT mice. Another point to be considered in diabetes models is that high IL-6 levels seem to be involved in insulin resistance found in obesity since IL-6 levels in adipose tissue correlated positively with adiposity. Also, weight loss seems to be associated with a reduction in the concentration of this cytokine [75,76]. In the current study, we found that despite the well-established weight gain observed in BACHD mice, the levels of IL-6 are significantly increased in the kidney and heart, but markedly decreased in the spleen. Our data suggest that other factors rather than obesity (or weight change) may underlie the changes in cytokine concentrations in peripheral organs observed in the BACHD mice. The local expression of mHTT in different tissues may explain, at least in part, the peripheral inflammatory profile found in the BACHD model.

Regarding IL-4, a regulatory cytokine known to be involved in adaptive immune response in HD [18], we found a significant increase in the spleen of BACHD mice. A previous study showed increased IL-4 plasma levels of HD patients at the moderate stage of the disease, suggesting that the increase of this cytokine may reflect an adaptive response to chronic immune activation [18]. The mechanisms involved in IL-4 signaling in the spleen during different stages of HD and its protective or deleterious role needs to be addressed.

The growing body of evidence regarding the involvement of the immune system in HD opened the road for the hypothesis that anti-inflammatory drugs or immunotherapies like active vaccination might be promising treatments for this currently incurable disease [5,6]. In this scenario, by employing both the R6/1 and the zQ175 knock-in mouse models of HD, an elegant study showed that active vaccination was able to modulate inflammatory/immune pathways in the spleen of immunized mice. A combination of three non-overlapping *HTT* Exon 1 derived peptides was used as the immunogen and a comprehensive and specific genome-wide RNA expression in the spleen of immunized mice was carried-out using microarrays. These authors found transcriptional activation of genes related with innate immune responses including TNF signaling pathways, transcriptional repression of genes associated with negative feedback mechanisms to control the expression of pro-inflammatory genes and with memory T cell responses [20]. These findings reinforce our data providing evidence of the role of peripheral organ-associated inflammatory pathways in HD and also highlight the potential of immune/inflammatory mechanisms as therapeutic targets.

## 5. Conclusion

Our main contribution here was to show preliminary evidence of a pro-inflammatory response or milieu in peripheral organs in HD (Fig.6). From an immunological perspective, HD pathogenesis must be investigated beyond the brain. Further studies evaluating the potential mechanisms and pathways of cytokine signaling in such organs are necessary and may contribute to identify peripheral biomarkers for HD and to broaden our knowledge regarding peripheral inflammatory responses in neurodegenerative diseases and their related syndromes.

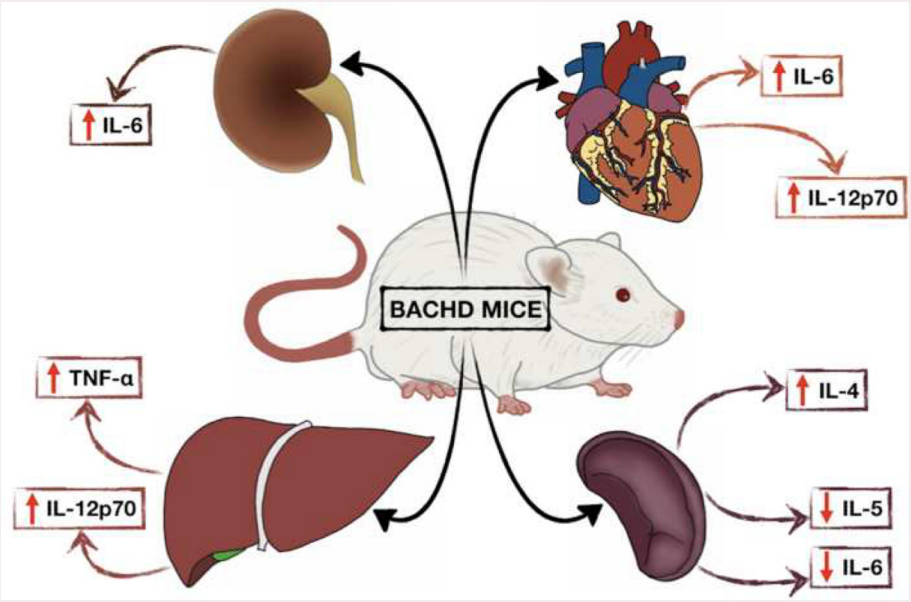


**Figure 6:.** Schematic representation of the study main findings.

Histopathological and functional analysis of the peripheral organs should also be carried out in order to reveal the impact of inflammation in peripheral tissue homeostasis and clinical outcomes in HD. Moreover, the current study was performed only in males. Immune response might be influenced by sex differences as well as by the reproductive cycle in females, and we recognize that relevant aspects should be further addressed in this regard.

## Data availability statement

All the data used to support the findings of this study are available from the corresponding author upon request.

## Declaration of Competing Interest

The authors declare no conflict of interest.

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