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Levo-1-methyl tryptophan aggravates the effects of mouse hepatitis virus (MHV-A59) infection

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ABSTRACT

Mice infected with mouse hepatitis virus A59 (MHV-A59) develop autoantibodies (autoAb) to liver and kidney fumarylacetoacetate hydrolase (FAH) with a concomitant enhancement of transaminases and release of alarmins such as uric acid and high-mobility group box protein 1 (HMGB1).
Tryptophan catabolism is an endogenous mechanism that restricts excessive immune responses, thereby preventing immunopathology. Since indoleamine-2,3-dioxygenase (IDO) is the key and rate-limiting enzyme of tryptophan catabolism, the aim of this work was to explore whether specific inhibition of IDO by Levo-1-methyl tryptophan (MT) could affect MHV actions.
Results showed that MT strongly enhanced the hypergammaglobulinemia induced by the virus, as well as anti-MHV Ab and uric acid release. Moreover, infected mice treated with MT did express anti-FAH autoAb and high levels of serum HMGB1. Survival of MHV-infected animals treated with MT was severely reduced compared with that of MHV-infected mice or controls only treated with MT. Furthermore, histological liver examination indicated that MT induced fibrosis in MHV-infected animals, whereas MT itself increased uric acid levels without shortening the animal life Thus, under our experimental conditions, results indicated an exacerbated response to MHV infection when IDO was blocked by MT.

1. Introduction

Mouse hepatitis virus strain A59 (MHV-A59) causes various mouse pathologies, including hepatitis, thymus involution [1], IgG2a-restricted hypergammaglobulinemia [2] and transient demyelination (Lavi et al., 1984) [3]. We have reported the presence of autoantibodies (autoAb) to fumarylacetoacetate hydrolase (FAH) in sera from various mouse strains after MHV-infection [4]. The autoAb recognized conformational as well as linear antigenic determinants in the enzyme, and the autoimmune response was partly related to molecular mimicry [5–7]. Furthermore, we have shown that the induction of the anti-FAH autoAb was associated with the MHV-induced release of some danger signals [8,9], also called DAMPs (damage-associated molecular patterns) [10]. Our results indicated an intimate association between the reactivity of autoAb to FAH and the presence of high levels of both uric acid and high-mobility group box protein 1 (HMGB1) in mouse serum [8]. We have suggested that the adjuvant effect of both alarmins, together with the fact that the FAH molecule shares some similarity with MHV proteins was implicated in the autoimmune response elicited by the viral infection [8].

Sustained access to nutrients is a fundamental biological need, especially for proliferating cells, and controlling nutrient supply is an ancient strategy to regulate cellular responses to stimuli. By catabolizing the essential amino acid tryptophan (TRP), cells expressing the enzyme indoleamine 2,3 dioxygenase (IDO) can mediate potent local effects on innate and adaptive immune responses to inflammatory insults [11]. IDO modifies immune responses in two ways: by producing kynurenine, a natural ligand for the aryl hydrocarbon receptor, and by depleting TRP to trigger aminoacid-sensing signal transduction pathways [11]. It has been reported that IDO acts also as a direct intracellular signaling molecule in dendritic cells (DCs) that express it [12].

IDO2 is a newly discovered enzyme with 43% similarity to classical IDO (IDO1) protein and shares the same critical catalytic residues. The role of IDO2 in human T cell immunity remains controversial, although it was demonstrated that, similar to IDO1, IDO2 also degrades TRP into kynurenine and is inhibited efficiently by Levo-1-methyl tryptophan (MT) [13]. In this paper, IDO refers to both enzymes, IDO1 and IDO2, since it is not possible to distinguish their activities.

IDO contains immunoreceptor tyrosine-based inhibitory motifs (ITIMs) that, once bound by distinct molecular partners, will either promote degradation or initiate signaling activity and self-maintenance of the enzyme [14]. Furthermore, altered IDO activity is often associated with pathology, including neoplasia and autoimmunity [15].

As IDO decreases the extent of the immune response, we wondered whether blocking the IDO activity could enhance the immune response

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and so protect the mice against the viral infection. Since another enzyme, tryptophan dioxygenase (TDO) is expressed primarily in liver where it catabolizes excess dietary TRP to maintain its serum concentrations below threshold levels, the reasoning for selecting L-MT instead of D-MT or the racemic mixture was that Pilotte et al. [16] demonstrated that L-MT inhibited IDO without affecting TDO.

To our surprise, we found that MT amplified the effects produced by MHV and strongly decreased animal survival. We also found that MT itself, in the absence of MHV infection, triggered the release of transaminases and uric acid.

2. Materials and methods

2.1. Mice

Specific-pathogen-free (SPF) female BALB/c mice from the University of La Plata, Argentina, were used at the age of 8–10 weeks. All animals were maintained in isolators, on standard laboratory chow, under SPF conditions until the end of the experiments, and received care in compliance with international legal requirements. Animals were grouped randomly and assigned to a specific experiment.

2.2. Preparation of MHV stock

The NCTC 1469 adherent cell line derived from normal mouse liver was purchased from the American Type Culture Collection. Cells growing in T-75 bottles were inoculated with MHV A59 virus at a multiplicity of 1–5 50% tissue infectious doses (TCID50) per cell. After an adsorption period of 1 h at 37 °C, 15 ml of NCTC 135 medium with 10% fetal calf serum was added to each bottle and incubated at 37 °C. Several cycles of freezing and thawing were used to release the virus 24 h after inoculation. The harvested virus was centrifuged at 400 *g* for 10 min to remove debris and the supernatant was frozen at −70 °C for storage. Virus titration by endpoint method was performed by inoculating serial dilutions of the MHV stock onto cell monolayers in 96-multiwell plates. After 24 h, wells with viral cytopathic effect were counted for each dilution and titer was expressed as TCID50 [4]. Before performing ELISA assays the virus was inactivated by incubating the MHV stock at 56 °C for 1 h [17].

2.3. Viral infection

Mice were inoculated intraperitoneally with 5×10^3 TCID50 of MHV A59 grown in NCTC 1469 cells [4] and bled at different times.

2.4. Treatments

Mice were given 1-L-methyl tryptophan (MT, Sigma-Aldrich Inc.,Illinois, MO), *ad libitum* in drinking water (5 g/l, pH 10.0, approximately 800 mg/kg/day), 72 h before being infected with MHV. MT was subsequently administered for 14 days and mice were bled at 7, 10, 14 and 28 days after MHV infection (mice called "MHV + MT"). As controls, other groups of mice were infected only with the virus ("MHV" mice), or treated with MT alone ("MT" animals).

2.5. Immunoglobulin (Ig) assays

For total Ig determination in mouse serum, microplates (Nunc Maxi-Sorb) were coated with 100 μl of phosphate buffer saline (PBS) containing a 1:500 diluted rabbit antiserum directed against mouse Ig. The plates were blocked for 1 h at 37 °C with 0.01 M Tris, 0.13 M NaCl, pH 7.4 (TMS) containing 5% of non-fat milk (TMS-M) and were incubated with serial dilutions of mouse serum in the same medium. After 2 h at 37 °C and washing with PBS containing 0.125 ml of Tween 20 per liter (PBS-Tween), the plates were incubated for 1 h at 37 °C with peroxidase-labeled goat directed against mouse IgG (Santa Cruz Biotechnology, CA) diluted 1:10,000 in TMS-M.

2.6. Determination of anti-MHV Ab by ELISA

To test anti-MHV Ab, ELISA plates were coated with 100 μl of UV-inactivated MHV-A59, 2×10^7 PFU/well, diluted in 0.02 M glycine, 0.03 M NaCl, pH 9.2. After overnight incubation at room temperature and washing with phosphate buffer saline containing 0.125 ml of Tween 20 per liter (PBS-Tween), the plates were blocked 2 h at 37 °C with 0.01 M Tris, 0.13 M NaCl, pH 7.4, containing 5% of fetal calf serum (TMS-FCS), which minimizes non-specific binding. The plates were then incubated 2 h at 37 °C with mouse serum diluted in TMS-FCS and after washing with PBS-Tween, the bound Ab were revealed with peroxidase-labeled goat anti-mouse IgG (Ig-PO, Santa Cruz Biotechnology, CA) diluted 1:10,000 in TMS-FCS. As a substrate, orthophenylenediamine-dihydrochloride (OPD, Sigma Chemical Co, St. Louis, MO) with freshly added H_2O_2 was used. The reaction was stopped after 10 min by addition of 1 M H2SO4. The absorption was measured by ELISA reader (Metertech Inc., Taipei, Taiwan) at 490 nm. Non-specific values of optical density were obtained in the absence of mouse serum.

2.7. Preparation of liver and kidney lysates

Livers and kidneys from control or treated BALB/c mice were removed, soaked in chilled PBS and ground in a Dounce homogenizer at 4 °C with 5 volumes of PBS containing 10^{-3} M phenylmethylsulfonyl fluoride (PMSF). The homogenates were centrifuged for 15 min at 400 ×*g* and the clarified extracts were kept at −20 °C until used. A sample of each suspension was solubilized by heating for 30 min at 100 °C in 1 M NaOH and protein concentration was determined by the method of Bradford [18].

2.8. Western-blot analysis

2.8.1. A) Determination of autoAb anti-FAH

Essentially, reactivity of autoAb anti-FAH was determined as indicated previously (Mathieu et al., 2001). Briefly, total liver or kidney extracts (100 μg of protein) were subjected to 10% SDS-PAGE and then transferred onto nitrocellulose sheets (GE Healthcare, Buckingghamshire, UK). The strips were incubated overnight at 4 °C with 30 mM Tris, 0.14 M NaCl, 0.1% (*v*/*v*) Tween 20, pH 8.0 (TBS-M-T) for 1 h at room temperature and the indicated serum dilution After several washings with TBS containing 0.1% Tween 20, bound Ab were revealed with peroxidase labeled goat against mouse IgG (Santa Cruz Biotechnology, CA, USA) diluted 1:10,000 in TBS-M-T and ECL plus reagents (GE Healthcare, Buckingghamshire, UK).

2.8.2. B) Determination of HMGB1 in serum

Mouse sera were filtered with Centricon YM-100 (Millipore Corp, USA) to clear the samples from macromolecular complexes, concentrated 15-fold with Centricon YM-30 and separated on 12% SDS-polyacrilamide gels. Western-blot analysis was carried out as described above, and HMGB1 was revealed with MAb anti-HMGB1 HAP46.5 (Santa Cruz Biotechnology, CA, USA) diluted 1:1000.

2.9. Serum uric acid determination

Uric acid concentration was determined enzymatically using the kit Uricostat (Wiener Lab, Rosario, Argentina) in 1:50 diluted mouse sera as indicated by the manufacturer.

2.10. Transaminase determination

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using GPT (ALT) or GOT (AST) Unitest (Wiener lab., Rosario, Argentina)

2.11. Histology

Livers from both control and treated mice ($n = 3$ for each condition) were removed, washed with PBS, cut into blocks and fixed by immersion into 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. After fixation, the tissues were dehydrated in graded alcohols and then embedded in paraffin. Five-micrometer tissue sections were cut, de-wax, hydrated and nuclei stained with Weigert's hematoxylin (BIOPUR, Rosario, Argentina) for 8 min. For collagen specific staining, sections were immersed in picro-sirius red solution, i.e., 0.1% Direct Red (Sigma-Aldrich Inc., Illinois, MO, USA), in picric acid saturated solution for 1 h. Stained sections were washed on 0.5% glacial acetic acid, dehydrated and mounted. Tissue images were obtained through an Axiolab Zeiss light microscope equipped with a cooled-digital camera Olympus QColor3.

2.12. Statistical analysis

Statistical significance between experimental values was calculated using Student's *t*-test. The Kaplan–Meier method was used to compare the differences in mouse mortality rates between groups. All statistical analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA).

3. Results

3.1. Total immunoglobulin (Ig)

As described before [2,4] "MHV" mice showed higher serum Ig levels than controls (Fig. 1). This hypergammaglobulinemia was still amplified in "MHV + MT" animals, mainly 7 days after infection and treatment (Fig. 1). By contrast, "MT" animals did not show any significant change in Ig concentration over controls at the three times of sampling (Fig. 1).

3.2. Serum aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) values in mice submitted to the various treatments

ALT occurs in high concentrations in the heart and liver with moderate amounts in skeletal muscle, kidneys, and pancreas, whereas AST is found in significant quantities in liver, kidney, and skeletal muscle, in decreasing order. As reported previously [9], we found that "MHV" animals exhibited elevated serum levels of AST after 14 and 28 days of treatment (Fig. 2). Results indicated that "MHV + MT" animals showed higher levels of the enzyme than "MHV" mice 10 days after treatment and infection, whereas MT treatment alone did not show any effect (Fig. 2). Analogous results were obtained for ALT determinations in all animals and times tested (data not shown).

3.3. Ab towards MHV

Anti-MHV Ab titers (expressed as the serum dilution to obtain an OD = 1.0) were significantly higher than controls 7 to 28 days post-infection in "MHV" animals (Fig. 3). It was observed that MT treatment strongly increased anti-MHV Ab in "MHV + MT" animals, mainly after 7–14 days of treatment, whereas values did not significantly differ from controls in "MT" mice (Fig. 3).

3.4. Uric acid concentration in serum

Because we have reported that mice infected with MHV showed high concentrations of both serum HMGB1 and uric acid [8], MHV-infected mice were treated with MT and the release of both alarmins was determined. It was found that both "MT" and "MHV + MT" animals showed a great increase of plasma uric acid, even over values obtained with "MHV" mice, mainly at 7 days post treatment (Fig. 4).

3.5. AutoAb to liver FAH and release of HMGB1

As stated above, MHV-infected mice had autoAb to liver and kidney FAH as detected by Western-blot assay. Present results indicated that after 14 days of treatment, MT administration did not show any effect on MHV-induction of autoAb to liver or kidney FAH (data not shown). Accordingly, results obtained 14 days after infection showed that the amount of HMGB1 in serum from "MHV + MT" animals was similar to that of "MHV" mice and that the solely MT administration did not induce the release of the protein (data not shown).

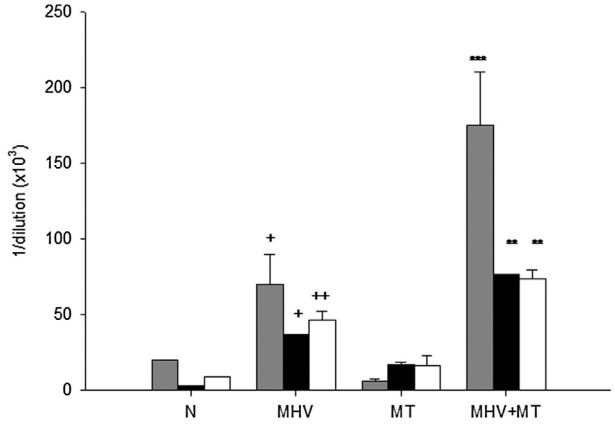

Fig. 1. Concentration of total Ig in sera from mice submitted to the indicated treatments. Results are the means of serum dilutions from 10 to 12 mice to reach an OD of 3.0 for 7 days (gray bars) and 0.8 for 14 (black bars) and 28 days (white bars) post infection and/or treatment, respectively. The tests were repeated at least three times with similar results. Statistical significance of values was determined by Student's *t* test in relation with MHV: $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, or in relation to *N* (control values): $^{+}P \leq 0.05$, $^{++}P \leq 0.01$.

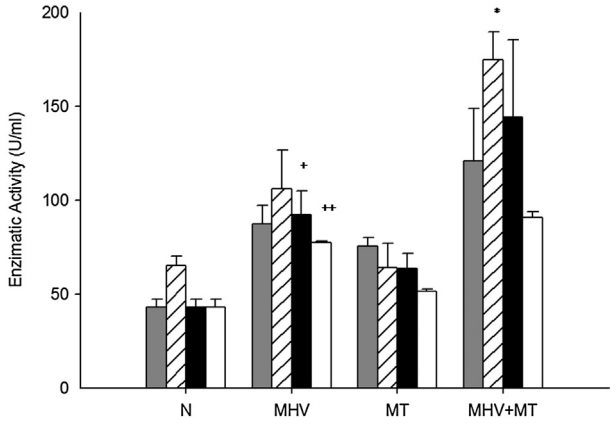

Fig. 2. Levels of serum transaminases in mice submitted to the indicated treatments. AST levels were determined in pools of sera from five animals bled after 7 (gray bars), 10 (hatched bars), 14 (black bars) or 28 days (white bars) post infection and/or treatment. N: normal or control mice (without any treatment). Results are means ± SE of three independent determinations. The tests were repeated at least three times with similar results. Statistical significance of values was determined by Student's *t* test in relation with MHV: $^{*}P \leq 0.05$, or in relation to *N* (control values): $^{+}P \leq 0.05$, $^{++}P \leq 0.01$.

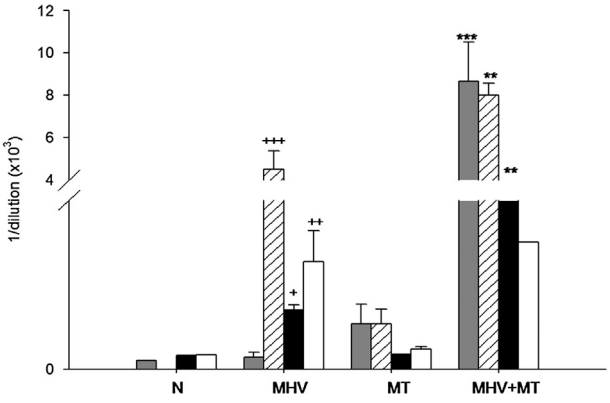

Fig. 3. Serum anti-MHV Ab in mice submitted to the indicated treatments. As described in Materials and methods, ELISA microplates were coated with inactivated MHV and incubated with diluted mouse serum corresponding to 7 (gray bars), 10 (hatched bars), 14 (black bars) or 28 days (white bars) post-infection and/or treatment. Bound Ab were detected with peroxidase-labeled goat anti-mouse IgG. Results are expressed as the mean serum dilution of pooled sera from 10 to 12 mice to reach an optical density value of 1.0 ± SE. The assays were repeated at least three times with similar results. N: normal or control mice (without any treatment). Statistical significance of values was determined by Student's *t* test in relation with MHV: $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, or in relation to *N* (control values): $^{+}P \leq 0.05$, $^{++}P \leq 0.01$, $^{+++}P \leq 0.001$.

3.6. Effect of MT treatment on survival of MHV-infected mice

Mouse survival was monitored daily for up to 4 weeks. Results showed that under our experimental conditions, neither MHV-infection ($n = 26$) nor MT treatment ($n = 20$) affected mouse survival during 28 days, the time of the assays (Fig. 5). By contrast, MT administration to MHV-infected mice ($n = 24$) reduced their life, the percent of survival being 60% at the end of the assays (Fig. 5). The Kaplan–Meier test was utilized to evaluate the differences in mortality rates between "MHV + MT" group in comparison with "MHV" and "MT" animals ($P \leq 0.001$) (Fig. 5).

3.7. Hepatic fibrosis

The liver responds to injury with a complex series of cellular events that result in accumulation of collagens, fibrosis, hepatocyte regeneration, nodule formation, altered blood flow, and cirrhosis [19]. Sirius red staining is used to reveal collagen fibers that appeared in red, whereas the rest of tissues are pale yellow [20]. Thus, we submitted the livers from control and treated and/or infected mice to Sirius red staining to examine the possible presence of histological fibrosis.

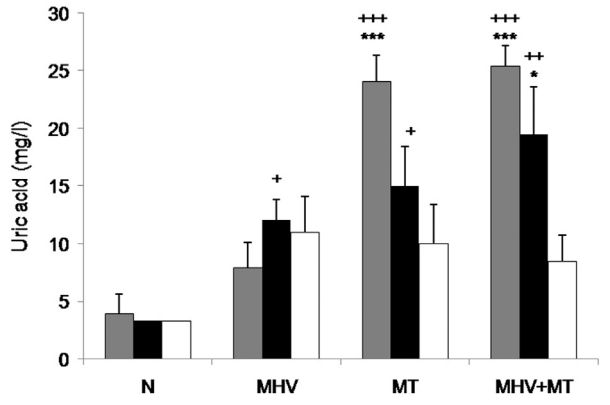

Fig. 4. Effect of MT on uric acid release induced by MHV infection. Mice were bled after 7 (gray bars), 14 (black bars) or 28 days (white bars) post infection and/or treatment. N: normal or control mice (without any treatment). Results are means ± SE of three independent determinations performed in each case with pooled sera from seven mice. Statistical significance of values was determined by Student's *t* test in relation with MHV: $^{*}P \leq 0.5$, $^{***}P \leq 0.001$, or in relation to *N* (control values): $^{+}P \leq 0.05$, $^{++}P \leq 0.01$, $^{+++}P \leq 0.001$.

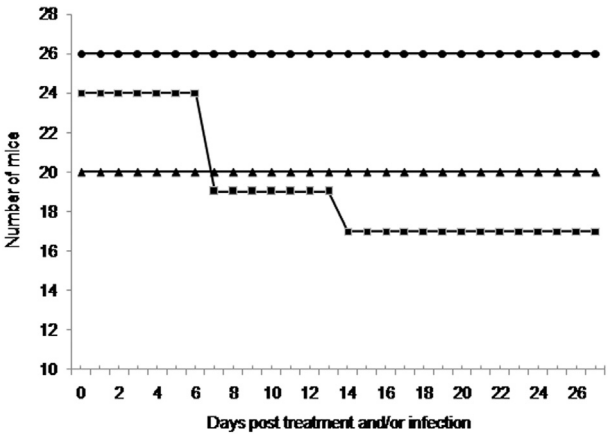

Fig. 5. Effect of MT administration on MHV-infected mouse survival. Data were taken from five independent experiments. Survival was monitored daily, for up to two weeks. A: "MHV" mice ($n = 26$) or "MT" animals ($n = 20$). B: "MHV + MT" mice ($n = 24$). The Kaplan–Meier test was utilized to compare the differences in mortality rates between groups. Significance compared with MHV or MT, $^{*}P \leq 0.001$.

Results indicated that livers from control, "MHV" and "MT" animals showed a very mild degree of fibrosis, compatible with an Ishak score of 0 (Fig. 6) [21]. By contrast, survival "MHV + MT" animals evidenced collagen accumulation indicating moderate to severe degree of fibrosis with occasional portal to portal bridging, suggesting of an Ishak score between 2 and 3 (Fig. 6) [20].

4. Discussion

It has been reported that signals that originate from dying cells, the damage-associated molecular patterns (DAMPs), determine apoptotic cell engulfment, dendritic cell (DC) activation, antigen processing, DC maturation and T cell activation [22,23]. It was suggested that structural features of autoAg as well as their locations, catabolism during cell death and translocation to cells that present antigens to the immune system could contribute to selection of the autoimmune repertoire [24]. In this way, we found that slow down of the release of uric acid and HMGB1 was able to reduce the autoimmune response to FAH elicited by MHV [8].

Tryptophan (TRP) is an essential amino acid for mammals. Tryptophan dioxygenase (TDO) is expressed primarily in liver where it catabolizes excess dietary TRP [16]. The other oxygenase/s, indoleamine 2,3 dioxygenase (IDO1 and IDO2) are expressed in a wider range of tissues [13]. Thus, the rationale for choosing L-MT instead of D-MT or the racemic mixture was that Pilotte et al. [16] demonstrated that L-MT inhibited IDO but not TDO.

IDO expression in certain antigen presenting cells (APCs), specifically some DC subsets, causes them to acquire potent and dominant regulatory functions that actively suppress T cell responses and promote T cell tolerance to further antigenic challenges [11]. Surprisingly, it was also suggested that IDO is not simply immunosuppressive, but rather plays a more complex role in modulating inflammatory responses, in particular those that are driven by autoreactive B cells [25].

The effect of MT treatment of MHV-infected mice showed in this work indicated that IDO inhibition seemed to exacerbate the signs of the viral infection. Thus, in the presence of MT, hypergammaglobulinemia as well as Ab titers to MHV and uric acid release enhanced even over values shown by "MHV" animals. Moreover, "MHV + MT" mice did express anti-FAH autoAb and high levels of serum uric acid and HMGB1 were also observed. It is tempting to relate all these finding to the fact that the solely MT administration strongly augmented the release of uric acid and

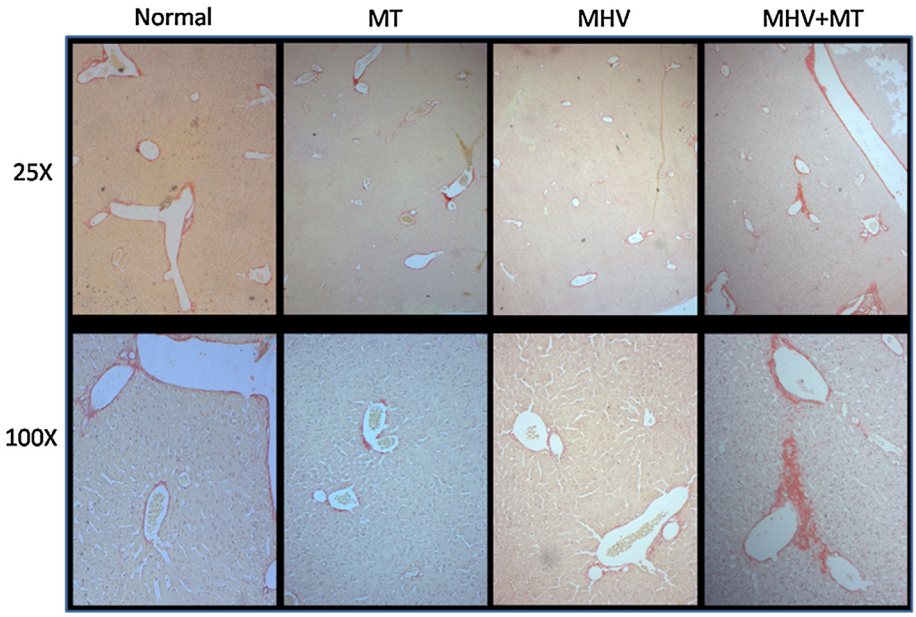

Fig. 6. Examples of liver fibrosis in animals submitted to the different treatments. Three livers from animals under each condition were excised. About ten samples from each organ were stained with Hematoxilyn and Sirius red. Collagen fibers are stained in red. Representative results after 14 days of treatment and/or infection are shown for three different livers under each treatment. Magnification: 25×, insert: 100×. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

transaminases, suggesting that the drug should enhance the cellular necrosis induced by the virus infection [26,27].

Hepatic fibrosis is the result of the wound-healing response of the liver to repeated injury. After an acute liver injury, parenchymal cells regenerate and replace the necrotic or apoptotic cells. This process is associated with an inflammatory response and a limited deposition of extracellular matrix proteins (ECM) [28]. If the hepatic injury persists, then eventually the liver regeneration fails, and hepatocytes are substituted with abundant ECM, including fibrillar collagen. The distribution of this fibrous material depends on the origin of the liver injury. In chronic viral hepatitis and chronic cholestatic disorders, the fibrotic tissue is initially located around portal tracts, while in alcohol-induced liver disease, it locates in pericentral and perisinusoidal areas. As fibrotic liver diseases advance, progression from collagen bands to bridging fibrosis to frank cirrhosis occurs [28].

We have found that MT augmented the mouse susceptibility to MHV-infection, since animal mortality increased under this compound administration. By contrast, "MT" animals behaved as controls. These observations are in close relationship with the finding of fibrosis in livers from survival "MHV + MT" animals, and not in "MT" mice. Unfortunately, livers from died mice could not be examined.

As explained above, IDO is considered an extrahepatic enzyme [11]. However, Larrea et al. [29] described the upregulation of IDO in the livers of patients and chimpanzees with chronic hepatitis C. Their findings indicated that viral infection facilitates the induction of IDO in response to proinflammatory cytokines and activated T cells, been probably a virus strategy to escape T-cell immunity [29]. Since MHV is a hepatotrophic virus, it is tempting to speculate that upregulation of IDO in livers of MHV-infected mice was induced under our experimental conditions. If this is the case, MT treatment should affect directly the liver enzyme activity leading to the enhancement of TRP catabolism and thus producing hepatic injury. Moreover, it was shown that HMGB1 expression in liver correlated with collagen deposition during hepatic fibrosis and that downregulating HMGB1 expression could exclude collagen production and enhance collagen degradation [30]. Thus, we cannot exclude this process as at least in part responsible for the development of liver fibrosis, since we found elevated levels of HMGB1 in serum of MHV-infected mice treated with MT.

Agaugué et al. [31] analyzed the direct effect of MT on the maturation of human monocyte-derived dendritic cells and showed that MT affected differentially the function of DC depending on the quality of the maturation signal, leading to either Th1 or Th2 responses. Interestingly, this effect of MT was not correlated to the inhibition of IDO activity [31]. Furthermore, it was shown that MT decreased antibody production after vaccination with hepatitis B surface antigen [32], whereas we found augmentation of Ab to MHV induced by the drug. Although Eleftheriadis et al. [32] do not discuss the effect of MT on DC, their results, as well as ours, may be related to the kind of Th responses induced by MT and not solely to its effect on IDO.

Because of the potent effector mechanisms of the immune system, the potential for self-destructive immune responses is especially high and many negative regulatory modalities exist to prevent excessive tissue damage [33]. In many cases, however, some pathological tissue damage is the necessary corollary of efficient immune responses, and when the infectious agents are controlled or the insult is removed, full tissue integrity and function are restored. Nevertheless, failure to properly regulate the response may result in more persistent pathological damage due to autoimmunity, inability to respond to concurrent infections, chronic inflammatory conditions, systemic metabolic syndrome, organ dysfunction due to inappropriate tissue repair, genetic instability and inflammation-induced carcinogenesis [34]. This recent concept, called *The price of immunity* is well exemplified in this work. Thus, mice were able to react against MHV attack; they were ill but eliminated the virus after a certain time. Similarly, although MT administration provoked some toxic effect, the animals survived. In those cases, the immune system worked at its best, without damaging the hosts. On the contrary, MT plus MHV-infection induced such a great inflammation and illness that mice could not afford that pathological stress and their immune system, to defend them, produced hepatic lesions detrimental for the animals.

TRP catabolism in cancer is increasingly being recognized as an important microenvironmental factor that suppresses anti-tumor immune responses, since TRP is catabolized in the tumor tissue by IDO and/or TDO, expressed in tumor cells [15]. This metabolic pathway creates an immunosuppressive milieu in tumors and in tumor-draining lymph nodes by inducing T-cell anergy and apoptosis through depletion of TRP and accumulation of immunosuppressive TRP catabolites. Competitive inhibitors of both enzymes are currently being tested in clinical trials in patients with solid cancer, with the aim of enhancing the efficacy of conventional chemotherapy [15,16].

Results presented in this work suggest that MT administration seemed to amplify some of the virus action, i.e., the hypergammaglobulinemia elicited by MHV, transaminases and uric acid release and autoAb to FAH, together with liver injury leading to fibrosis and eventually cirrhosis. Thus, although other viral infections must be studied, our observations suggest that MT should be kept away from treating people under viral infections, mainly hepatotropic agents.

Disclosure of potential conflict of interest

No conflict of interest was disclosed by the authors.

Acknowledgments

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