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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.