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ORIGINAL ARTICLE

Mast cells' involvement in inflammation pathways linked to depression: evidence in mastocytosis

S Georgin-Lavialle1,2,3,20, DS Moura1,2,4,20, A Salvador5,6, J-C Chauvet-Gelinier7,8, J-M Launay9, G Damaj10, F Côté2, E Soucié11, M-O Chandesris1, S Barète1,2, C Grandpeix-Guyodo1, C Bachmeyer3, M-A Alyanakian12, A Aouba13, O Lortholary1,14, P Dubreuil1,11, J-R Teyssier15, B Trojak6,7, E Haffen15,16,17, P Vandel17,18, B Bonin7,8, the French Mast Cell Study Group21, O Hermine1,2,13,22 and R Gaillard1,5,6,19,22

Converging sources of evidence point to a role for inflammation in the development of depression, fatigue and cognitive dysfunction. More precisely, the tryptophan (TRP) catabolism is thought to play a major role in inflammation-induced depression. Mastocytosis is a rare disease in which chronic symptoms, including depression, are related to mast cell accumulation and activation. Our objectives were to study the correlations between neuropsychiatric features and the TRP catabolism pathway in mastocytosis in order to demonstrate mast cells' potential involvement in inflammation-induced depression. Fifty-four patients with mastocytosis and a mean age of 50.1 years were enrolled in the study and compared healthy age-matched controls. Depression and stress were evaluated with the Beck Depression Inventory revised and the Perceived Stress Scale. All patients had measurements of TRP, serotonin (5-HT), kynurenine (KYN), indoleamine 2,3-dioxygenase 1 (IDO1) activity (ratio KYN/TRP), kynurenic acid (KA) and quinolinic acid (QA). Patients displayed significantly lower levels of TRP and 5-HT without hypoalbuminemia or malabsorption, higher IDO1 activity, and higher levels of KA and QA, with an imbalance towards the latter. High perceived stress and high depression scores were associated with low TRP and high IDO1 activity. In conclusion, TRP metabolism is altered in mastocytosis and correlates with perceived stress and depression, demonstrating mast cells’ involvement in inflammation pathways linked to depression.

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INTRODUCTION

Converging sources of evidence point to a role for inflammation in the development of depression, fatigue, and cognitive dysfunction.1–6 The major role of proinflammatory cytokines has been well characterized, allowing to demonstrate that they interact with multiple pathways known to be involved in the development of depression, including monoamine metabolism, neuroendocrine function, synaptic plasticity and neurocircuits relevant to mood regulation. Studying patients treated with interferon alpha has been very fruitful in this regard.7 Besides, cell-mediated immune activation has been highlighted in depression, with an emphasis on activated T lymphocytes, peripheral macrophages, microglial, astroglial cells and their interactions with glial cells.8–10 The roles of other immune cells are underexplored. We focus here on mast cells, whose residence in perivascular locations on the brain side of the blood–brain barrier indicates that they are strategically situated to initiate microglial, neural and vascular responses.11–13 Mast cells are

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innate immune cells involved in homeostasis maintenance and immune response regulation. Mast cell activation induces the release of various molecules contained in their cytoplasmic granulations, such as histamine, serotonin (5-HT), tryptase, prostaglandin, cytokines and chemokines. They are able to secrete proinflammatory cytokines such as tumor necrosis factor α (TNFα), interleukin (IL)-1, IL-6 and IL-18. Indeed they sit at major inflammation pathways linked to depression.

Table 1. Main features of the study population (N = 54)

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>50.1</td>
<td>50.0</td>
</tr>
</tbody>
</table>

**Clinical forms**

- No. cutaneous: 2 (3.7%)
- No. systemic indolent: 46 (85.2%)
- No. systemic aggressive: 4 (7.4%)
- No. SM-AHNMD: 2 (3.7%)

**Genotype**

- No. D816V: 41 (75.9%)
- No. WT: 9 (16.7%)
- No. missing: 4 (7.4%)

**Biology**

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptase (μL)</td>
<td>101.3 (183.6)</td>
<td>4.1 (0.8)</td>
</tr>
<tr>
<td>Tryptophan (μmol L⁻¹)</td>
<td>41.7 (10.8)</td>
<td>53.0 (2.6)</td>
</tr>
<tr>
<td>No. low tryptophan (&lt;45)</td>
<td>32 (59.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Kynurenine (μmol L⁻¹)</td>
<td>3.1 (0.8)</td>
<td>3.4 (0.7)</td>
</tr>
<tr>
<td>Serotonin (μmol)</td>
<td>284.4 (249.6)</td>
<td>388.2 (124.5)</td>
</tr>
<tr>
<td>No. low serotonin (&lt;150)</td>
<td>18 (33.3%)</td>
<td>2 (3.7%)</td>
</tr>
<tr>
<td>IDO1 (%)</td>
<td>8.1 (3.4)</td>
<td>6.4 (1.3)</td>
</tr>
<tr>
<td>No. high IDO1 (&gt;7.5)</td>
<td>26 (48.1%)</td>
<td>19 (18.5%)</td>
</tr>
<tr>
<td>Quinolinic acid (μmol L⁻¹)</td>
<td>571.9 (133.0)</td>
<td>473.4 (76.2)</td>
</tr>
<tr>
<td>Kynurenine (μmol L⁻¹)</td>
<td>39.0 (9.2)</td>
<td>35.1 (4.1)</td>
</tr>
<tr>
<td>IFN-γ (μmol L⁻¹)</td>
<td>0.42 (0.17)</td>
<td>0.15 (0.07)</td>
</tr>
</tbody>
</table>

**Psychology**

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beck depression inventory</td>
<td>16.5 (12.1)</td>
<td>–</td>
</tr>
<tr>
<td>No. BDI ≥ 10</td>
<td>36 (66.7%)</td>
<td>–</td>
</tr>
<tr>
<td>No. BDI ≥ 19</td>
<td>16 (29.6%)</td>
<td>–</td>
</tr>
<tr>
<td>Perceived stress</td>
<td>38.3 (13.8)</td>
<td>–</td>
</tr>
<tr>
<td>No. stress ≥ 40</td>
<td>25 (46.3%)</td>
<td>–</td>
</tr>
</tbody>
</table>

Abbreviations: BDI, beck depression inventory; IDO1, indoleamine 2,3-dioxygenase 1; IFN-γ, interferon gamma; SM-AHNMD, systemic mastocytosis with associated hematological non mast cell disease; WT, wild type.

TRP, 5-HT, KYN, KA and QA measurements

Blood samples were collected in 7-ml Vacutainer (Becton-Dickinson, Le Pont de Claix, Isère, France) collection tubes containing heparin or EDTA. Except for 5-HT measurement, the tubes were centrifuged at 2500 g and 4°C for 15 min, and the separated plasma was aliquoted in 0.5-ml fractions into 1.5-ml polypropylene vials (Corning, NY, USA), and then frozen at −80°C until analysis.

Plasma TRP and whole-blood 5-HT rates were determined by high-performance liquid chromatography using the method of Kema et al. KYN and KA were measured using high-performance liquid chromatography as described by Fujii et al. and Swartz et al., respectively, whereas QA was measured using mass fragmentography. The KYN/TRP ratio was calculated from absolute concentrations of KYN and TRP and was used as an index of IDO1 activity. We compared levels of 5-HT, TRP, IDO1, KYN, KA and QA between the 54 patients and 54 age-matched healthy controls (with neither psychological affection nor mastocytosis) (Table 1). Taking as thresholds the 90th percentiles of a large (n = 416) control cohort, TRP was considered low when its rate was <45 μM; IDO1 activity was considered high if >7.5% and 5-HT levels were considered low if under 150 nM.

Plasma interferon gamma (IFN-γ) was measured using an electrochemiluminescent immunoassay (MSD, Rockville, MD, USA).

The detection limits, and intra- and inter-assay variabilities can be found in Supplementary Table S1.

**MATERIALS AND METHODS**

**Patients**

Adults with a diagnosis of mastocytosis (N = 54) and a mean age of 50.1 years were enrolled in a prospective multicentric French study between 2007 and 2011 (Table 1). They consisted of 66.7% women. All patients provided their informed consent. The study was approved by the ethical committee at Necker hospital, and was carried out in compliance with the precepts of the Helsinki protocol. To establish the diagnosis of mastocytosis, following international WHO criteria, we provided measurements of tryptase serum level as previously described, as well as those obtained during a search for KIT gene mutations in the skin and/or bone marrow. Indolent forms of mastocytosis were present in 90.9% of patients (46 ISM, 2 CM) and aggressive forms in 11.1% (4 aggressive systemic mastocytosis, 2 systemic mastocytosis with associated hematological non mast cell disease). The median tryptase level in patients was 31.9 ng L⁻¹ (mean 101.3 ng L⁻¹), significantly higher than controls (P = 0.0001). The D816V genotype was present in 75.9% of patients (n = 41).

**Psychological analysis**

Depression and stress. Participants completed the French version of Beck Depression Inventory (BDI-II), a 21-item self-report questionnaire designed to measure the severity of depression symptoms. Total score indicates the severity of participant's depression symptoms: doubtful (0–9), mild (10–18), moderate (19–29) or severe (30–63). To consider patients as depressed a score of 10 was adopted as the cutoff point; to discriminate patients with mild and moderate–severe depression, the cutoff score of 19 was adopted. Stress was assessed by the Perceived Stress Scale, which measures the degree to which life events are appraised as stressful by individuals.

As mastocytosis represents a paradigm of mast cell activation, we decided to study a cohort of 54 patients with proven mastocytosis (N = 54) and compared patients' levels of TRP, 5-HT, KYN, IDO1 activity, KA and QA with the same metabolites and enzyme levels in healthy control subjects (N = 54). Our study would pinpoint mast cells' involvement in inflammation pathways linked to depression.

**Statistical analysis**

Two-tailed Wilcoxon matched pairs tests and correlations by the linear regression method were performed using GraphPad Prism software version 5.01 (GraphPad Software, San Diego, CA, USA) and R statistical software (www.r-project.org). Considering the preferential involvement of QA in depression pathophysiology in comparison with KA, one-tailed Wilcoxon test was performed to compare QA and KA patient/control ratios.
RESULTS

Main neuropsychological features
Among the 54 patients with mastocytosis, mean depression score was 16.5 (range = 0–45) and 66.7% of patients displayed a depression score ≥10, including 20 mild-depression patients and 16 moderate-to-severe-depression patients (Table 1). Perceived stress in our sample was high (mean = 38.3; range = 37–63), with 46.3% of patients displaying a score ≥40.

Higher IDO1 activity and lower levels of TRP and 5-HT in mastocytosis
In our study cohort, 59.3% (n = 32) of patients presented abnormally low levels of TRP, 48.1% (n = 26) high IDO1 activity and 33.3% (n = 18) low 5-HT levels. None of our patients displayed malabsorption, especially neither severe digestive mastocytosis, which could explain low TRP absorption, nor hypoalbuminemia. Patients displayed lower levels of TRP and 5-HT than controls (respectively, P < 0.0001 and P < 0.0001) and higher IDO1 activity (P < 0.01) and higher levels of KA and QA (respectively, P < 0.01 and P < 0.0001) (Figure 1). KYN was significantly lower among patients (P < 0.05). In order to compare KA and QA pathways, we computed the difference (ratio) between patients and controls in the KA/KYN ratio and compared it with the difference between patients and controls in the QA/KYN ratio. KYN metabolism was preferentially oriented towards QA, with an increase in the QA/KYN ratio in patients that was significantly larger than the increase in the KA/KYN (P < 0.05).

Patients with high perceived stress and high depression scores present high IDO1 activity and low TRP
Whole-blood 5-HT levels did not correlate with depression score (P > 0.8). Instead, depression score negatively correlated with plasma levels of TRP (P ≤ 0.0001) and positively correlated with plasma IDO1 activity (P ≤ 0.0001) (Figure 2). Neither correlation was found with plasma KA or QA levels.

Neither age nor BMI correlated significantly with IDO1, TRP or BDI (all P > 0.4). Adding age and BMI as independent variables in these correlations indicated that keeping age and BMI constant, depression (BDI scores) still correlated negatively with TRP (P < 0.0001) and positively with IDO1 (P < 0.0001). An interaction analysis using BMI category (according to BMI median split) showed no interaction between BMI category and these correlations (P > 0.25). A similar interaction analysis using age category (according to age median split) showed that the slopes of the correlation lines with depression for the group of older patients was significantly smaller that that for the younger patients for TRP (P < 0.05) and marginally so for IDO1 (P = 0.083). Crucially, negative correlation between depression and TRP and positive correlation between depression and IDO1 remained significant in each age group (P < 0.0001 and P < 0.0001 in older patients, and P < 0.01 and P < 0.05 in younger patients).
IFN-γ is considered to be the most potent inducer of IDO1,41 and was found to be higher in patients than in controls in our study (P < 0.0001). We therefore evaluated the effect of IFN-γ on the above results. IFN-γ did not correlate with depression and IDO1 (P > 0.5). Moreover, introducing IFN-γ as a cofactor in the correlation analyses did not alter the correlations between depression and TRP or IDO1, which remained significant (both P < 0.0001). Therefore, the correlations found between BDI and TRP and IDO1 do not appear to be mediated by IFN-γ.

We compared depressed patients (score ≥ 10, n = 36) with non-depressed patients (n = 18), and patients with high perceived stress (scores ≥ 40, n = 25) with patients with low perceived stress in regard to 5-HT, TRP, KA and QA. We first noted that there was some correlation between high depression scores and high stress scores, with depressed patients presenting significantly higher levels of perceived stress (mean = 43.0 versus 29.1; P < 0.01) and high-stress patients presenting higher levels of depression (mean = 23.5 versus 10.5; P < 0.01). With regard to biological measures, depressed patients presented significantly lower plasma TRP levels than controls (mean = 39.7 versus 45.6; P < 0.05). Patients with high perceived stress displayed significantly higher levels of plasma IDO1 activity (mean = 9.5 versus 6.9; P < 0.01) and lower plasma TRP levels (mean = 37.2 versus 45.6; P < 0.01). No significant difference was found for plasma KYN or KA or QA levels (all P > 0.2).

**DISCUSSION**

In this study we aimed to demonstrate mast cells’ potential involvement in inflammation pathways linked to depression pathophysiology. Mastocytosis, a rare disease with mast cell accumulation and activation, provided us a unique condition to reach this goal. Depression and cognitive impairment are frequent and associated with disability in patients with mastocytosis.19–21 In a large cohort of patients with mastocytosis, we focused on a physiological pathway: TRP metabolism. We demonstrated that patients displayed significantly higher plasma IDO1 activity, higher levels of plasma KA and QA, and lower levels of plasma TRP and whole-blood 5-HT as compared with healthy individuals. Moreover, IDO1 activity and TRP levels correlated with perceived stress and depression scores.

These results are reminiscent of previous studies performed in patients with inflammation-induced depression. The role of proinflammatory cytokines such as interferon alpha, TNF or IL-6 has been repeatedly demonstrated among interferon-alpha-treated patients, in depression with elevated C-reactive protein level, and in experimental proinflammatory challenges such as endotoxin injection or vaccine.42–51 Inflammation pathways include TRP metabolism through IDO1 activation, leading to abnormal accumulation of KYN catabolites linked to depressive symptoms.1,8 Few articles have focused on cells involved in these cascades. Besides activated T lymphocytes or peripheral macrophages and microglial changes, few data are available regarding the role of mast cells.18,1632 Here, our results on the study of TRP metabolism in a rare condition, mastocytosis characterized by mast cell accumulation and activation, and a high prevalence of depression and cognitive impairment, suggest that directly or indirectly mast cells may be involved in psychiatric manifestations of inflammation.17,19

The brain contains numerous mast cells, which are located along vessels, preferentially near the blood–brain barrier on the side of the brain.12 Mast cells are also located in high density in the hypothalamus, which is involved in systems of stress response, emotion and cognition, as well as in amygdalas and the thalamus, lesions or stimulation of which have been associated with changes in emotional reactivity and pain.3–30 Because of their specific distribution and density in the brain, mast cells’ over-activity may interfere with brain function and adversely affect stress response, cognition and emotionality.16,57–60 Mast cells can secrete proinflammatory cytokines such as TNFs, IL-1 and IL-6, and are able to express IDO1.14 Moreover, it has recently been shown that KYN catabolites are able to activate mast cells in mastocytosis are able to induce the enzyme indoleamine-2,3-dioxygenase (IDO1) that catalyzes TRP into kynurenine (KYN) and then quinolinic acid, thereby reducing the availability of TRP for 5-HT synthesis via the enzyme TRP hydroxylase. The activation of the KYN pathway may also be due to the induction of the liver enzyme TRP dioxygenase by increased cortisol secretion in case of stress.

Figure 3. Hypothetical tryptophan (TRP) metabolism in mastocytosis. Cytokines (interleukins 1 and 6, tumor necrosis factor α) synthetized by activated mast cells in mastocytosis are able to induce the enzyme indoleamine-2,3-dioxygenase (IDO1) that catalyzes TRP into kynurenine (KYN) and then quinolinic acid, thereby reducing the availability of TRP for 5-HT synthesis via the enzyme TRP hydroxylase. The activation of the KYN pathway may also be due to the induction of the liver enzyme TRP dioxygenase by increased cortisol secretion in case of stress.
according to ClinicalTrials.gov) to restore immune functions against cancer cells.72

Cholinergic blockade by KA, a KYN catabolite, could also be solved by alpha7 nicotinic acetylcholine receptor agonists.73 This strategy is also in line with the currently approved vagus nerve stimulators in depression.74 We observed a significant imbalance towards QA in comparison with KA. This last result is in line with the NMDA receptor agonist effect of QA leading to neurotoxic effects via lipid peroxidation, and disruption of the blood–brain barrier, while KA might have neuroprotective effects.75–77 As stressed on by Capuron and Miller,78 ketamine, an NMDA antagonist, allows a rapid and dramatic improvements in mood in patients with treatment-resistant depression and could target inflammation-induced depression.76–79 Given the preferential increase of QA levels in comparison with KA levels in mastocytosis patients, this therapeutic strategy might be efficient in mastocytosis patients displaying stress and depression.

Finally, as we demonstrated here mast cells’ potential involvement in inflammation-induced depression, clinical trials could be performed in the near future in order to test the efficacy of molecules that target mast cells, such as tyrosine kinase inhibitors (Figure 3).20,79

CONFLICT OF INTEREST

RG has received compensation as a member of the scientific advisory board of Janssen, Lundbeck, Roche, Takeda. He has served as consultant and/or speaker for Astra Zeneca, Pierre Fabre, Lilly, Otsuka, SANOFI, Servier and received compensation, and he has received research support from Servier. AS has consulted for Servier and received compensation. SGL has served as consultant and/or speaker for SOBI and Novartis and received financial help from SOBI and Bayer for travelling to congress. OH received research funding and honorarium from AB Science. The remaining authors declare no conflict of interest.

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FRENCH MAST CELL STUDY GROUP


AUTHOR CONTRIBUTIONS


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