Indoleamine 2,3-dioxygenase is increased in hemodialysis patients and affects immune response to hepatitis B vaccination

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1. Introduction

Many clinical and experimental studies indicate that acquired immunity is impaired in hemodialysis (HD) patients. Facts like the poor response to vaccination against hepatitis B virus (HBV), the high frequency of negative delayed type hypersensitivity skin tests, the increased risk for active tuberculosis and the high failure rates of vaccination with T-cell dependent peptide antigens but not with T-cell independent polysaccharide antigens confirm that T-cell function is impaired in this population [1,2].

Experimental studies showed that the interaction between antigen presenting cells (APCs) and T-cells is impaired in HD patients. Staphylococcal enterotoxin B (SEB)-induced ligation between major histocompatibility complex (MHC) molecules and T-cell receptors (TCRs) leads to reduced T-cell proliferation and ζ-chain phosphorylation, one of the first events in TCR-derived signal transduction pathway [3]. Impaired immune synapse function due to decreased MHC and TCR expression, altered expression of the adhesion molecules lymphocyte function antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1), and reduced expression of the co-receptors CD80/CD86 in APCs and CD28 in T-cells could contribute to the acquired immunity disturbances in this population [4–7].

Besides the impaired APC-T-cell interaction other mechanisms have been also proposed for the acquired immunity disturbances in HD patients. Increased soluble CD40 molecules, which impair the interaction between T-cells and B-cells [8], decreased TCR ζ-chain expression [9], altered calcium kinetics in lymphocytes [10], altered cytokine status, which promotes differentiation of naïve CD4+ lymphocytes to helper T-cells type 1 (Th1) [11], and increased T-cells and B-cells apoptosis [12,13] have all been incriminated for the impaired adaptive immune response in HD patients. The research in the field is intensive and continuous.

Indoleamine 2,3-dioxygenase (IDO) is a 45 kDa enzyme that catalyses the initial rate-limiting step of tryptophan degrada-
tion along the kynurenine pathway. IDO is inducible by various inflammatory stimuli, mainly by interferon-γ (IFN-γ), but also by IFNs type I, tumor necrosis factor-α (TNF-α), and lipopolysaccharide (LPS). This enzyme is widely distributed in various cell types, the APCs, monocytes, macrophages and dendritic cells (DCs) included. Its expression in APCs is accompanied by impaired adaptive immune response because tryptophan depletion and kynurenine pathway products in local microenvironment decrease T-cell proliferation, increase T-cell and B-cell apoptosis and induce the emergence of regulatory T-cells (Tregs) from naïve T-cells [14–16]. IDO mediated immunosuppression reduces transplantation rejection [17–19], and ameliorates the clinical course of various experimental autoimmune diseases [20–22].

Inhibition of T-cell function via IDO is also mediated by non-APC cell types. Expression of IDO in paternally derived placental trophoblast contributes to success of semi-allogenic pregnancy [23,24], while its expression in lung epithelial cells inhibits helper T-cell type 2 (Th2) response in an experimental model of asthma [25]. In renal transplant rejection, besides infiltrating mononuclear cells, IDO is also expressed in tubular epithelial cells, possibly as an unsuccessful in this case counteracting mechanism to local inflammation [26]. Finally, IDO expressed by tumor cells contributes to escape of tumors by immunosurveillance [27].

The role of IDO in acquired immunity disturbances in HD patients has not been evaluated yet. Although there are studies that claimed increased IDO activity in the serum of HD patients, these studies evaluated this activity very indirectly by estimating the serum kynurenine to tryptophan ratio [28–30]. However, such an approach is inaccurate because it does not consider the possible low tryptophan serum levels in case of malnutrition, the decreased renal excretion of kynurenine pathway products and the activity of the liver tryptophan 2,3-dioxygenase, which is known to be upregulated in experimental models of renal failure [31,32].

In the present study we compared plasma IDO concentration between HD patients and healthy volunteers. In order to evaluate the role of IDO in acquired immunity disturbances in HD patients we evaluated if it affects their immune response to HBV vaccination. The inflammatory markers C-reactive protein (CRP), interleukin-6 (IL-6) and TNF-α were also evaluated in the serum because inflammation usually accompanies HD, has been incriminated for the impaired adaptive immune response in this population, and interacts with IDO [19,14–16]. Finally, serum albumin was also assessed in HD patients as a marker of nutrition, which is downregulated by inflammation [33,34].

2. Patients and methods

2.1. Patients

Sixty-six stable HD patients (44 males, mean age 61.06 ± 12.66 years) and twenty-four healthy volunteers derived from two HD units personnel (12 males, mean age 57.0 ± 8.61 years) participated in the study. The two groups did not differ significantly regarding the cause of end stage renal disease (diabetes mellitus in 22 patients, primary glomerulonephritis in 19 patients, obstructive nephropathy in 4 patients, hypertension in 4 patients, interstitial nephritis in 4 patients, autosomal dominant polycystic kidney disease in 3 patients, Alport’s syndrome in 1 patient and unknown in 9 patients).

According to the literature, all the HD patients were initially vaccinated with four double doses (40 mcg) of recombinant HBV vaccine (Engerix, GlaxoSmithKline, Rixensart, Belgium), intramuscularly at 0, 1, 2 and 6 months. All doses of the vaccine were repeated in patients who had not responded 1–2 months after complete first vaccination series. Nevertheless, only one boost dose was being administered in patients with initial antibody against the HBV surface antigen (anti-HBs) levels greater than 10 IU/L, due to successful response to vaccination who then presented with reduced anti-HBs levels [35–39]. After the initial determination, 1–2 months after the first four vaccine doses completed, anti-HBs antibodies were routinely determined every 6 months. All patients enrolled into the study had the first four vaccine series completed and none of them had ever naturally been infected with HBV as indicated by negativity for antibodies against the HBV core antigen (anti-Hbc).

Patients underwent regular HD with polysulfone low-flux dialyzers and bicarbonate buffer for 4 h sessions, 3 times a week. Medical history, physical examination, routine laboratory tests and imaging studies excluded patients with infection, malignancy or autoimmune disease. Finally, none of them was receiving cytotoxic drugs, corticosteroids or non-steroid anti-inflammatory drugs. An informed consent was obtained from each individual enrolled into the study and the hospital ethics committee gave its approval to the study protocol.

3. Methods

Blood samples were drawn just before the start of the second HD session of the week. The samples were centrifuged immediately and the harvested plasma and serum were stored at −20 °C.

Serum anti-HBs antibody levels were determined by means of chemiluminescent microparticle immunoassay (CMIA) using the ARHITECT i2000SR automatic analyzer (Abbott Laboratories, Abbott park, IL, USA). Plasma IDO was measured with an ELISA kit (CUSABIO Biotech Co., Wuhan, China). The analytical limit of detection of the above ELISA kit is 0.195 ng/ml. The proinflammatory cytokines IL-6, and TNF-α were measured in the serum by means of ELISA using two commercially available kits (Biosource Europe S.A., Nivelles, Belgium). Serum CRP was measured using the COBAS INTEGRA 400 automatic analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Serum albumin was also assessed in HD patients.

3.1. Statistical analysis

The normal distribution of the variables was evaluated using the one-sample Kolmogorov–Smirnov test. For comparison of means between HD patients and healthy volunteers or between patients with adequate and patients with inadequate immune response to HBV vaccination unpaired t-test or Mann–Whitney U-test were used as appropriate. Results were expressed as mean ± SD and were considered as statistically significant when two-sided p < 0.05. Median values and ranges were also calculated (and are provided in the tables and figures of the present manuscript). For evaluating correlations among variables, Pearson’s r or Spearman’s Rho were calculated as appropriate. Finally, Fisher’s exact test was used for evaluating associations between categorical variables.

4. Results

4.1. Plasma IDO and serum CRP, IL-6, and TNF-α levels in HD patients and healthy volunteers

Compared to healthy volunteers, plasma IDO concentration was twice higher in HD patients (44.68 ± 31.90 ng/ml vs. 22.17 ± 25.98 ng/ml, p = 0.001, unpaired t-test) (Fig. 1). CRP was also increased in HD patients (9.84 ± 14.41 mg/L vs. 3.51 ± 0.99 mg/L, p = 0.001, Mann–Whitney U-test). Both evaluated proinflammatory cytokines were very increased in HD patients.

Serum IL-6 was 14.90 ± 12.42 pg/ml in HD patients and only...
2.02 ± 2.01 pg/ml in healthy volunteers (p < 0.001, unpaired t-test). Serum TNF-α was 14.47 ± 5.21 pg/ml in HD patients and 4.55 mg/L vs. 10.52 ± 16.03 mg/L, p = 0.476, Mann–Whitney U-test). Both evaluated proinflammatory cytokines did not differ significantly between those HD patients with adequate immune response and those with inadequate immune response. Serum IL-6 was 11.74 ± 5.30 pg/ml in HD patients with adequate immune response and 15.75 ± 5.27 pg/ml in HD patients with adequate immune response (p = 0.095, unpaired t-test). Serum TNF-α was 12.44 ± 4.60 pg/ml in HD patients with adequate immune response and 15.02 ± 5.27 pg/ml in HD patients with adequate immune response (p = 0.101, unpaired t-test). Serum albumin did not differ between patients with adequate or inadequate immune response to HBV vaccination (3.98 ± 3.13 g/dl vs. 3.99 ± 0.18 g/dl respectively, p = 0.893, unpaired t-test) (Table 2).

4.2. Plasma IDO and serum CRP, IL-6, and TNF-α levels in HD patients with adequate or inadequate response to HBV vaccination

At the time of the study, from the sixty-six patients, fourteen (21.21%) did not develop adequate anti-HBs antibody levels (>10 IU/L). The two groups of patients did not differ significantly regarding age (60.19 ± 13.02 vs. 64.29 ± 11.05 years, p = 0.286, unpaired t-test).

Compared to HD patients with adequate immune response to HBV vaccination, plasma IDO concentration was significantly higher in HD patients with inadequate immune response (67.68 ± 33.73 ng/ml vs. 38.50 ± 28.69 ng/ml, p = 0.002, unpaired t-test) (Fig. 2).

CRP did not differ significantly between the two patients’ groups (7.32 ± 4.55 mg/L vs. 10.52 ± 16.03 mg/L, p = 0.476, Mann–Whitney U-test). Both evaluated proinflammatory cytokines did not differ between those HD patients with adequate immune response and those with inadequate immune response. Serum IL-6 was 11.74 ± 5.30 pg/ml in HD patients with adequate immune response and 15.75 ± 5.27 pg/ml in HD patients with adequate immune response (p = 0.095, unpaired t-test). Serum TNF-α was 12.44 ± 4.60 pg/ml in HD patients with adequate immune response and 15.02 ± 5.27 pg/ml in HD patients with adequate immune response (p = 0.101, unpaired t-test). Serum albumin did not differ between patients with adequate or inadequate immune response to HBV vaccination (3.98 ± 3.13 g/dl vs. 3.99 ± 0.18 g/dl respectively, p = 0.893, unpaired t-test) (Table 2).

Interestedly, in the patients’ group IDO was negatively correlated with all markers of inflammation, i.e., CRP (Rho = -0.348, p = 0.004), IL-6 (r = -0.300, p = 0.015) and TNF-α (r = -0.317, p = 0.010). CRP was positively correlated with IL-6 (Rho = 0.607, p < 0.001) but negatively with albumin (Rho = -0.440, p < 0.001). IL-6 was also negatively related to albumin (r = -0.265, p = 0.032), which was the case for TNF-α as well (r = -0.239, p = 0.05). On the contrary, IDO was positively related to albumin (r = 0.427, p < 0.001) (Table 3).

IDO levels did not differ between diabetic and non diabetic HD patients and were 37.29 ± 34.02 ng/ml and 48.39 ± 30.52, respectively (p = 0.185, unpaired t-test). Additionally, from the 22 diabetics inadequate immune response to HBV vaccination was observed in 4 patients, while from the rest 44 HD patients inadequate immune response to HBV vaccination was observed in 10 patients. Diabetes mellitus did not affect the adequacy of the immune response to HBV vaccination (p = 0.759, Fisher’s exact test).

5. Discussion

In the present study plasma IDO concentration and its impact on acquired immune response in HD patients were evaluated. Numerous clinical and experimental studies indicate that adaptive immune response is impaired in this population [1]. One of the clinical data that supports the above is the impaired development of protective antibodies after vaccination against HBV. Indeed, despite the administration of double vaccine doses and of an extra dose in HD patients, response rate is about 50–60%, while the response rate in the general population is higher than 90% [35,36]. At the time of the study and despite the rigorous effort for achieving successful immunization against HBV, less than 80% of our patients had adequate anti-HBs levels (>10 IU/L).

Table 1

<p>| Markers of inflammation in HD patients and healthy volunteers. Compared to healthy volunteers, serum levels of CRP, as well as serum levels of the proinflammatory cytokines IL-6, and TNF-α were significantly higher in HD patients. |</p>
<table>
<thead>
<tr>
<th>HD patients</th>
<th>Healthy volunteers</th>
<th>p</th>
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<tbody>
<tr>
<td>CRP (mg/L)</td>
<td>9.84 ± 14.41</td>
<td>3.51 ± 0.99</td>
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<tr>
<td>Median: 4.7 range: 72.1</td>
<td>Median: 3.0 range: 2.9</td>
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</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>14.90 ± 12.42</td>
<td>2.02 ± 2.01</td>
</tr>
<tr>
<td>Median: 10.0 range: 53.3</td>
<td>Median: 2.2 range: 6.7</td>
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<tr>
<td>TNF-α (pg/ml)</td>
<td>14.47 ± 5.21</td>
<td>6.46 ± 0.59</td>
</tr>
<tr>
<td>Median: 14.2 range: 19.7</td>
<td>Median: 6.2 range: 1.7</td>
<td></td>
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</tbody>
</table>

Fig. 1. IDO levels in HD patients and healthy volunteers. Compared to healthy volunteers, plasma IDO concentration was twice higher in HD patients (44.68 ± 31.90 ng/ml vs. 22.17 ± 25.98 ng/ml, 95% CI of deference from 9.26 ng/ml to 35.77 ng/ml, p = 0.001, unpaired t-test) (left panel). The median IDO concentration was 48.9 ng/ml (range 112.8 ng/ml) in HD patients and only 8.0 ng/ml (range 75.5 ng/ml) in healthy volunteers (right panel).
Markers of inflammation in HD patients with adequate or inadequate immune response to HBV vaccination program. Serum albumin, CRP, as well as the proinflammatory cytokines IL-6, and TNF-α did not differ significantly in HD patients with adequate or inadequate immune response to HBV vaccination.

Table 2

<table>
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<th>Adequate immune response (anti-HBs &gt; 10 IU/L)</th>
<th>Inadequate immune response (anti-HBs &lt; 10 IU/L)</th>
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</thead>
<tbody>
<tr>
<td>CRP (mg/L)</td>
<td>10.52 ± 16.03</td>
<td>7.32 ± 4.55</td>
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<tr>
<td>IL-6 (pg/ml)</td>
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</tr>
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<td>Albumin (g/dl)</td>
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<td>3.99 ± 0.18</td>
<td>0.893</td>
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</tbody>
</table>

In order to investigate new factors that could affect adaptive immunity in HD patients, we evaluated IDO concentration in their plasma. Compared to healthy volunteers, IDO concentration was twice higher in HD patients. CRP, IL-6 and TNF-α were also much higher in HD patients than in the control group, confirming previous studies which showed that chronic renal failure, HD procedure or both could be considered as inflammatory conditions [1,9]. The reason for IDO overexpression, reflected on its plasma levels in the present study, in HD patients needs further evaluation. However, because it is known that IDO is upregulated by various inflammatory stimuli [14–16], it is possible that its overexpression in HD patients results from the chronic inflammation that characterizes them.

After confirming the elevated plasma levels of IDO in HD patients, we evaluated its possible contribution to the known impaired adaptive immune response in this population. Indeed, compared to HD patients with adequate immune response to HBV vaccination program, plasma IDO concentration was almost twice higher in HD patients with inadequate immune response. Taking into consideration the immunosuppressive properties of IDO [14–25,27], it is likely that IDO contributes to the impaired adaptive immune response in HD patients.

On the contrary, chronic inflammation did not affect the immune response to HBV vaccination in the cohort of our patients. We did not find significant differences in the inflammatory markers CRP, IL-6, and TNF-α and in albumin in HD patients with adequate or inadequate immune response to HBV vaccination. This is in accordance with other studies [40,41]. There is discrepancy about the effect of age on the immune response to HBV vaccination [40,41]. However, the lack of difference in age in our study could be simply the result of the few young participants. Peces et al showed that HD patients younger than 40 years have a great opportunity for a successful immune response to HBV vaccine [40]. From our patients only four were younger than 40 years. Interestingly, the existence of diabetes mellitus also did not have an impact on immune response to HBV vaccination. Although the markers of inflammation were increased in diabetics (data not shown) IDO levels did not differ between diabetic and non diabetic HD patients. It is possible that chronic inflammation does not affect adaptive immunity directly, but through the induction of other mediators, such as IDO.

As expected, in the patients’ group CRP was positively correlated with IL-6, since the last is the major cytokine that induces CRP production by the liver [42], but negatively with albumin. IL-6 and TNF-α were also negatively related to albumin. These results are in accordance with the known negative impact of chronic inflammation on nutritional status and albumin levels in HD patients [33,34].

In the patients’ group IDO was negatively correlated with all evaluated markers of inflammation, i.e., CRP, IL-6, and TNF-α. On the contrary, IDO was positively related to albumin. At first glance

Table 3

<table>
<thead>
<tr>
<th></th>
<th>CRP</th>
<th>IL-6</th>
<th>TNF-α</th>
<th>Albumin</th>
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<td>IDO</td>
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<td>r = −0.300 (p = 0.015)</td>
<td>r = −0.317 (p = 0.010)</td>
<td>r = 0.427 (p &lt; 0.001)</td>
</tr>
</tbody>
</table>

Fig. 2. IDO levels in HD patients with adequate or inadequate immune response to HBV vaccination program. Compared to HD patients with adequate immune response to HBV vaccination (anti-HBs > 10 IU/L), plasma IDO concentration was significantly higher in HD patients with inadequate immune response (anti-HBs < 10 IU/L) [67.68 ± 33.73 ng/ml vs 38.50 ± 28.69 ng/ml, 95% CI of difference from 11.27 ng/ml to 47.10 ng/ml, p = 0.002, unpaired t-test] (left panel). The median IDO concentration was 38.6 ng/ml (range 89.5 ng/ml) in HD patients with adequate immune response to HBV vaccination and 74.0 ng/ml (range 112.8 ng/ml) in HD patients with inadequate immune response (right panel).
these findings seem to be unexpected. However, it is possible that after its initial induction by inflammation, upregulated IDO curtails its own provoking agent, i.e., inflammation.

Positive feedback loops able to enhance IDO expression have been identified. Induction-induced IDO expression in APCs converts naïve T-cells to Tregs [14–16]. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) in Tregs interacts with B7 antigen in APCs and induces IDO expression in the last cells [43]. Experimentally, another positive feedback loop has been revealed in CD19+ plasmacytoid DCs (pDCs). Tryptophan depletion activates the general control nonrepressed 2 (GCN2) kinase, which is required for expression of IFN-α after B7 ligation, promoting through the last cytokine further the production of IDO [43,45].

Then upregulated IDO could ameliorate inflammation by diminishing oxidative stress through superoxide anion consumption or the generation of kynurenine pathway products that are avid oxygen scavengers [46,47]. Also, in pDCs activated GCN2 kinase due to IDO-induced tryptophan depletion phosphorylates ribosomal eukaryotic translation initiation factor 2α (eIF2α) altering the translation of NF-IL-6 and inhibiting the transcription of IL-6, which is necessary for the conversion of Tregs to proinflammatory helper T-cells type 17 (Th17) [48].

In conclusion, IDO is increased in HD patients. It is possible that after its initial upregulation due to chronic inflammation, IDO curtails its own provoking agent, i.e., inflammation. Increased IDO suppresses adaptive immunity in HD patients, as it is assessed by the immune response to HBV vaccination.

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[11] Sester U, Sester M, Hauk M, Kaul H, Kohler H, Girndt M. T cell activation follows the generation of kynurenine pathway products that are avid oxygen scavengers [46,47]. Also, in pDCs activated GCN2 kinase due to IDO-induced tryptophan depletion phosphorylates ribosomal eukaryotic translation initiation factor 2α (eIF2α) altering the translation of NF-IL-6 and inhibiting the transcription of IL-6, which is necessary for the conversion of Tregs to proinflammatory helper T-cells type 17 (Th17) [48].


