

Increased Indoleamine 2,3-Dioxygenase in Monocytes of Patients on Hemodialysis

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Hemodialysis patients suffer from susceptibility to infections. Inflammation upregulates indoleamine 2,3-dioxygenase (IDO) in the antigen-presenting cells, which suppresses T-cell function. Plasma IDO activity or protein expression is increased in hemodialysis patients and is associated with immune disturbances. This observation, however, does not consider many factors, importantly the source of IDO, which has to be the antigen-presenting cells in order IDO to exert its immunosuppressive effect in the microenvironment of the immune response. In this study, monocytes were isolated from 30 hemodialysis patients and 20 healthy volunteers and IDO was assessed by Western blotting. The IDO level in the monocytes of hemodialysis patients was significantly, almost 3-fold, higher than in the monocytes of healthy volunteers. This localization enables IDO to exert its immunosuppressive effect and supports conclusions of previous studies that used more indirect methods for assessing the role of this enzyme in the context of the immune response in hemodialysis patients.

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Uremic toxins and hemodialysis procedure per se induce inflammation and disturbances of adaptive immunity, increasing the susceptibility to infections and reducing the response to vaccines.¹ Because infections are a major cause of morbidity and the second cause of death in hemodialysis patients,² discovering the pathogenetic mechanisms involved in the immune deficiency that characterize this population is of great importance.

Indoleamine 2,3-dioxygenase (IDO) is expressed, especially under inflammatory conditions, in antigen presenting cells (APC), degrades L-tryptophan along the kynurenine pathway, and suppresses T-cell function.³ Indoleamine 2,3-dioxygenase has been studied in hemodialysis patients and has been associated with inflammation, atherosclerosis, and inadequate response to vaccination.⁴⁻⁶ Several studies evaluated the plasma IDO activity by

measuring the kynurenine to L-tryptophan ratio,^{4,5} or less frequently plasma IDO concentration.⁶ However, the 1st approach is inaccurate due the low tryptophan levels in case of malnutrition, the decreased renal excretion of kynurenine, and the activity of the liver tryptophan 2,3-dioxygenase, which is upregulated in kidney failure.^{7,8} Assessing plasma IDO protein level does not discriminate an increased expression in APC form an increased APC turnover. In order to resolve these problems, we evaluated IDO expression in the monocytes of hemodialysis patients.

After approval from the hospital's ethics committee, 30 hemodialysis patients and 20 age-matched healthy volunteers were enrolled in the study. The patients were on regular hemodialysis, 3 times a week for 58.7 ± 40.6 months, with polysulfone low-flux dialyzers. None of them

suffered from active infection, malignancy, or active autoimmune disease, or was a carrier of hepatitis B, hepatitis C, or human immunodeficiency virus. None of the patients were receiving corticosteroids or cytotoxic drugs for at least 1 year prior to the study and none of them received a blood transfusion for at least 6 months prior to the study.

Peripheral blood mononuclear cells were isolated from whole blood by Ficoll-Hypaque density gradient centrifugation (Histopaque 1077, Sigma-Aldrich, St Louis, MO, USA). After washing with RPMI 1640 (Sigma-Aldrich), mononuclear cells were seeded in 6 flat-bottom well plates and incubated for 1 hour at 37°C in a humidified atmosphere containing 5% carbon dioxide. Nonadherent lymphocytes were discarded and the cells were incubated for another hour. After a 2nd removal of the remaining nonadherent lymphocytes, adherent monocytes were lysed using the T-PER tissue protein extraction reagent (Pierce Biotechnology, Rockford, IL, USA) supplemented with protease and phosphatase inhibitors (Sigma-Aldrich).

Protein was quantified via Bradford assay (Sigma-Aldrich), and equal quantities (10 µg) from each sample were used for Western blotting. Blots were incubated with the primary rabbit polyclonal immunoglobulin G anti-IDO antibody sc-25809 (Santa Cruz Biotechnology, Dallas, TX, USA) for 16 hours, followed by secondary antibody (Anti-rabbit immunoglobulin G, HRP-linked Antibody, Cell Signaling Technology, Danvers, MA, USA) for 30 minutes. Analysis was performed using the Image J software (National Institute of Health, Bethesda, MD, USA). In order to compare optical densities between bands in different lanes, a common control sample was loaded in all gels and the results were expressed as the ratio of the optical density of each band to the optical density of the band corresponding to the control sample.

Western blotting analysis revealed that compared to healthy volunteers, IDO expression in monocytes of hemodialysis patients was significantly, almost 3-fold, increased ($P < .001$). More precisely, the mean IDO expression ratio was 3.04 ± 1.78 in the hemodialysis patients and 1.14 ± 0.48 in the healthy volunteers. Figure 1 depicts 2 representative IDO Western blotting lanes, and Figure 2 depicts the difference in IDO expression in the monocytes between the hemodialysis patients and the healthy volunteers.

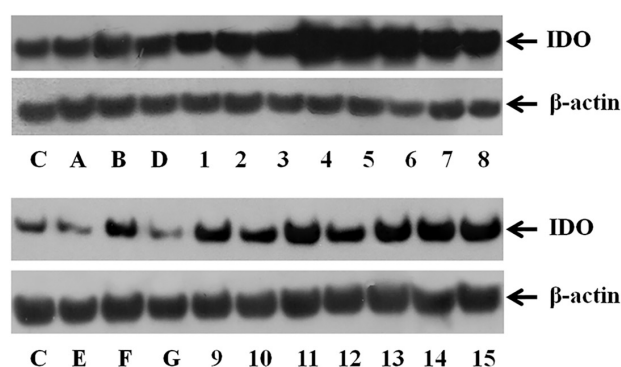


Figure 1. Indoleamine 2,3-dioxygenase expression in the monocytes of hemodialysis patients and healthy volunteers. Monocytes were isolated from the blood of hemodialysis patients and healthy volunteers and Western blotting was performed in the extracted protein. Two representative lanes are depicted. Capital letters correspond to healthy volunteers, whereas numbers correspond to hemodialysis patients. Note that a sample from the same healthy volunteer (C) is present in both lanes. Indoleamine 2,3-dioxygenase quantity for each sample was calculated as the optical density of each band divided by the optical density of the band corresponding to the control sample C.

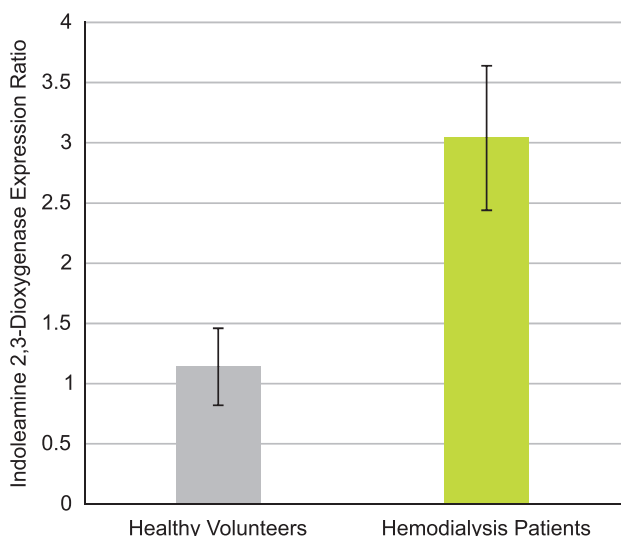


Figure 2. Indoleamine 2,3-dioxygenase expression was markedly increased in the monocytes of hemodialysis patients.

Since infections contribute significantly to the morbidity and mortality of hemodialysis patients,² efforts to understand the pathogenesis of the acquired immunity disturbances, which characterize these patients,² is of great interest. Indoleamine 2,3-dioxygenase is a known immunosuppressive enzyme.³ To date, it is thought that IDO is increased in hemodialysis patients by studies that assessed its plasma activity or its plasma concentration. They associated increased IDO in hemodialysis patients with inflammation, atherosclerosis, and impaired adaptive immune response.⁴⁻⁶ However,

as already has been noted, severe limitations exist regarding the interpretation of these results in the context of an immune response. Impaired adaptive immune response takes place in secondary lymphoid tissue, in a microenvironment that allows close interaction between T cells and APC, which under inflammatory conditions, expresses high levels of IDO.³ In the present study, we assessed IDO expression in the monocytes of hemodialysis patients in order to resolve this problem. In the context of an immune response, monocytes are transformed to APC. Interestingly, in hemodialysis patients monocytes are activated.¹

Indoleamine 2,3-dioxygenase exerts its effect on T cells through L-tryptophan depletion and activation of general control nonderepressible 2 kinase,⁹ or through activation of aryl-hydrocarbon receptor by kynurenine.¹⁰ However, in the above studies, a lower concentration of L-tryptophan or a higher concentration of kynurenine were required for suppression of T-cell function than those observed in the plasma of hemodialysis patients.^{4,5} Confirmed by the present study, increased IDO expression in the APC of hemodialysis patients favors a role of this enzyme in the immunodeficiency state that characterizes this population, since it permits severe L-tryptophan depletion and high kynurenine concentrations in the microenvironment where the immune response takes place.

Currently, because IDO is upregulated in case of inflammation,³ which is common in hemodialysis patients,¹ efforts to constrain inflammation may improve acquired immune response. Also, studies with relatively nontoxic inhibitors of IDO or of downstream molecules, such as aryl-hydrocarbon receptor, might be proved clinically useful in the future.^{11,12}

In conclusion, IDO is increased in monocytes of hemodialysis patients. This localization enables IDO to exert its immunosuppressive effect and supports conclusions of previous studies that used more indirect methods for assessing the role of this enzyme in the context of the immune response in hemodialysis patients.

CONFLICT OF INTERESTS

None declared.

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