Interferon-γ Mediated Pathways And Mitogen Stimulated Proliferation During And After An Acute Infection

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Abstract:
Background: Interferon-γ (IFN-γ) regulates the degradation of tryptophan to kynurenine via induction of indoleamine-2,3-dioxygenase (IDO). Local tryptophan depletion and accumulation of toxic metabolites might impair the proliferative capacity of lymphocytes. The aim of this study was to assess the actual status of immune system activation of patients with bacterial infection in the acute phase and during convalescence in vivo and in vitro. Parameters of systemic immune system activation were evaluated for associations with proliferative responsiveness of immune cells, and compared with healthy controls.

Methods: 24 patients with various acute bacterial infections were included in the group of acutely ill patients. Sixteen patients participated in a follow-up examination after convalescence. The control group consisted of 6 healthy people. To assess the status of immune system activation in vivo, inflammation parameters C-reactive protein and differential blood counts were determined. Neopterin concentrations were measured by enzyme-linked immunosorbent assay (ELISA). Tryptophan and kynurenine measurements were performed with high pressure liquid chromatography (HPLC). Peripheral blood mononuclear cells (PBMCs) were isolated from the patients’ blood and stimulated with concanavalin A (Con A), phytohemagglutinin (PHA) and pokeweed mitogen (PWM)

Results: Patients with acute bacterial infections showed reduced tryptophan and elevated neopterin concentrations, which did not normalize after convalescence period. Higher plasma neopterin values and increased IDO-activity were associated with reduced proliferative responses in vitro after stimulation with PHA. Associations were observed during acute infection as well as convalescence.

Conclusions: Results of this study show that increased immune system activation in vivo is associated with impaired proliferative responsiveness of immune cells in vitro in acute bacterial infections as well as during convalescence.

Keywords: neopterin; bacterial infection; proliferative response.

Introduction

The pteridines neopterin and 7,8-dihydronopterin are formed from guanosine triphosphate (GTP) by the enzyme GTP-cyclohydrolase I [1]. Due to a relative deficiency of 6-pyrovoyl tetrahydropterin synthase, primate macrophages and monocytes form neopterin derivatives upon stimulation with IFN-γ instead of, as in other species, bioterins [1,2]. IFN-γ is the major stimulus [3], but also tumor necrosis factor-α (TNF-α) and lipopolysaccharide (LPS) can contribute to neopterin production [4]. During inflammatory processes, macrophages and T-cells produce large amounts of reactive oxygen species (ROS) [5]. Depending on the pH value and the ratio of neopterin to 7,8-dihydronopterin, neopterin derivatives modulate the cytotoxicity of macrophages and interfere in the redox
equilibrium [5,6]. Neopterin and 7,8-dihydroneopterin can either increase or diminish the oxidative potential, thus they act not only as markers of oxidative stress, but contribute to the modulation themselves [5,6]. During acute viral infections, plasma and urine neopterin values increase markedly, which is already assessable at the end of an incubation period and before the onset of symptoms [1,7]. After seroconversion, neopterin values decrease again and reach normal values when the pathogen is competed [1,7]. In acute bacterial infections, neopterin values remain relatively low, as humoral immunity is predominating, which makes neopterin a marker of differentiation between acute bacterial and viral infections [1,7]. Septic patients have been shown to have higher concentrations of neopterin compared to patients with systemic inflammatory response syndrome due to infection [8]. The ratio of CRP to neopterin (C/N) was found to have a high sensitivity and specificity to discriminate between bacterial and viral respiratory infections [9]. In different cellular immunity involving processes, high neopterin values can be determined. Accordingly, patients with malignancies [10], cardiovascular diseases [11] and neurodegenerative conditions, such as Alzheimer’s disease [12], present with elevated neopterin concentrations.

As a marker for IFN-γ induced Th-1-type immune response, neopterin is strongly related to tryptophan metabolism. Tryptophan is an essential amino acid and is therefore necessary for the biosynthesis of proteins. The two enzymes tryptophan-2,3-pyrrolase (TDO) and indoleamine-2,3-dioxygenase (IDO) metabolize tryptophan via the so-called kynurenine pathway to produce quinolinic acid and nicotinamide [13,14]. Both catalyze the oxidative degradation of tryptophan to N-formylkynurenine [14,15]. While TDO is mainly expressed in the liver, IDO induction was detected in several types of cells as antigen presenting cells including macrophages, monocytes, dendritic cells and fibroblasts [14-17]. Interferon-γ (IFN-γ) acts as the most potent stimulator of IDO [13,15,16,18-21]. Normally, the degradation by TDO predominates, but under pathologic conditions, IDO activity exceeds tryptophan catabolism via TDO [15]. Strongly enhanced tryptophan degradation is especially seen in patients with increased T-cell activation, as this increased degradation occurs during infections, autoimmune diseases, neurodegenerative processes and coronary heart disease [18,22-25]. To differentiate low tryptophan plasma levels due to enhanced degradation from tryptophan deficiencies, the ratio of kynurenine to tryptophan (kyn/trp) can be calculated [15]. In cases of poor tryptophan intake, kynurenine values would also be low, in contrast to high ratios due to increased catabolization. A high kyn/trp-ratio in combination with high values of neopterin or IFN-γ activity implicates IDO induction and immune activation triggered tryptophan degradation [15].

Limited tryptophan availability in the local micromilieu results in the inhibition of protein synthesis and proliferation in pathogens and tumor cells [26-29]. Tryptophan shortage and the resultant accumulation of toxic kynurenine metabolites also suppresses T-cell proliferation [27]. Placental macrophages ensure maternal acceptance of the fetus through T-cell modulation by induction of IDO expression [20]. This also constitutes a feedback mechanism to prevent overshooting immune reactions [19]. High neopterin values and enhanced IDO activity in chronic immune system activation have been associated with suppressed proliferation rates of lymphocytes [30]. In this study, proliferative responses of lymphocytes to mitogen stimulation in vitro have been examined for associations with neopterin plasma concentration and IDO activity.

Materials and Methods

Study population

The study group consisted of 24 acutely ill patients (15 female, 9 male, mean age 64 ± 4.75 years) with a variety of bacterial infections (15 patients with infections of the respiratory system, 4 patients with urinary tract infections, 3 patients with infective gastroenteritis, one patient with erysipelas, one patient with kidney abscess). Sixteen patients (7 female, 9 male) participated in a follow-up examination after convalescence (mean age 56 ± 5.77 years). The control group included 6 healthy controls (mean age 52 ± 7.68 years).

Informed consent: Written informed consent was obtained from all patients according to the declaration of Helsinki.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the University’s local ethics committee.

Assay Methods

Parameters of immune system activation

Blood samples of healthy controls were taken once. Blood samples of acutely ill patients were taken at the
time of infection and after convalescence as a follow-up examination. CRP, lymphocyte counts and segmented neutrophils as well as the immune activation parameters neopterin, tryptophan and kynurenine were measured in the plasma.

**PBMC Cultures**

PBMCs were isolated from whole blood obtained from healthy blood donors (control group) as well as from patients with various acute bacterial infections during and after infection. Cells were separated by density centrifugation after supplementation with PBS and Biocoll separation solution (MedPro, Vienna, Austria). The separated cells were washed thrice with PBS with 0.3%-EDTA (0.5 mmol/L) and seeded at a density of 1x10^6 after supplementation with 10% heat-inactivated fetal calf serum (Invitrogen, Lofer, Austria), 2mM L-glutamine (Serva, Heidelberg, Germany) and 50 μg/ml gentamycin (Serva, Heidelberg, Germany) in RPMI-1640-medium (MedPro, Vienna, Austria). The cells were stimulated with the mitogens concanavalin A (Con A), phytohemagglutinin (PHA) and pokeweed mitogen (PWM; all from Sigma-Aldrich, Vienna, Austria) and incubated at 37°C in 5% CO₂ for 48 hours. After this period, cells were separated by centrifugation and the concentrations of neopterin, tryptophan and kynurenine were measured in the supernatants.

**Measurement of Neopterin, Tryptophan and Kynurenine**

Neopterin concentrations were measured by ELISA (BRAHMS, Hennigsdorf, Germany). To determine IDO activity, tryptophan and kynurenine concentrations were measured with HPLC (high-performance liquid chromatography) at a UV-absorption of 366 and 360 nm. 3-nitro-L-tyrosin (Sigma-Aldrich, Vienna, Austria) was used as an internal standard. Kyn/trp was calculated by means of the determined concentrations of kynurenine and tryptophan and expressed as μM/mM. CRP/Neopterin ratio (C/N) was also calculated.

**Cell Proliferation**

Cell proliferation was measured by ³H-thymidine incorporation. After a 48 hour stimulation period 1 μCi of ³H-thymidine and 10 μl of medium were added to the cells and incubated for another 18 hours. Thymidine incorporation was measured by a liquid scintillation counter in counts per minute (cpm).

**Statistical Analysis**

The statistical analysis of the data was performed with IBM SPSS Statistics 24 (IBM Corp., Armonk, NY). As data did not show normal distribution, non-parametric tests were applied. For comparisons of grouped data, a Mann-Whitney-U Test was performed for independent and Wilcoxon for paired samples. Correlations were examined with spearman rank correlation analysis. P-values < 0.05 were considered as significant.

**Results**

**Parameters of Immune Activation**

Inflammatory parameters such as CRP, segmented neutrophils and lymphocyte counts were elevated in the group of acutely ill patients (data shown in Table 1). Concentrations of neopterin were significantly elevated in acutely ill patients (p=0.002) and convalescents (p=0.03) compared with healthy controls. Neopterin tended to be higher in acutely ill patients than during convalescence (p=0.053). Tryptophan plasma concentrations were significantly higher in healthy controls than in acutely ill (p=0.011) and recovered patients (p=0.027). Higher neopterin plasma concentrations were seen in patients with lower tryptophan (rs=-0.636, p=0.001) and higher kyn/trp (rs=0.816, p<0.001), indicating that tryptophan degradation was immune-mediated.

The C/N-Ratio was elevated in acutely ill patients compared with convalescents (p=0.001) as well as with healthy controls (p=0.004). During acute infection, patients over the age of 65 tended to show lower tryptophan concentrations (p=0.068) and higher kyn/trp (p=0.059) than younger individuals. After convalescence, higher values of neopterin (p=0.006), kynurenine (p=0.059) and kyn/trp (p=0.009) were observed in patients over the age of 65 compared to younger patients. During acute infection, older age correlated with lower tryptophan concentrations (rs=-0.422; p=0.045). Older age was also significantly associated with plasma neopterin (rs=0.602; p=0.014) and kyn/trp (rs=0.605; p=0.013) after convalescence. In patients with acute infections, neopterin was associated with lower tryptophan (rs=-0.636; p=0.01), kynurenine
(rs=0.681; p<0.001) and kyn/trp (rs=0.816; p<0.001). High neopterin concentrations were associated with decreased lymphocyte counts in acutely ill patients (rs= -0.481; p=0.032). In the acute phase of infection, male patients had significantly higher tryptophan concentrations than women (p=0.012). No other differences in vivo or in vitro were found when comparing male and female patients.

### PBMC Proliferation and Its Relationship to Parameters of Immune Activation

Proliferation rates in unstimulated PBMCs of acutely ill patients were higher than during convalescence (p=0.036). After stimulation with Con A, cells of acutely ill patients proliferated better than cells of healthy controls (p=0.014). When cells were stimulated with PHA, the highest proliferation rates were seen in healthy controls (not significant), and PBMCs of acutely ill patients less than 65 years old proliferated better than cells of older patients (p=0.039).

Older age was associated with lower proliferative responses to Con A in the acute phase (rs= -0.522; p=0.015) and PHA in acutely ill (rs= -0.438; p=0.047) as well as in recovered patients (rs= -0.682; p=0.01).

During acute infection, proliferative responses upon PHA stimulation strongly correlated with concentrations of neopterin (rs= -0.591; p=0.005) and tryptophan (rs= -0.505; p=0.019). High C/N-Ratios were tendentially associated with worse proliferation rates upon stimulation with PWM in acutely ill patients (rs= -0.427; p=0.06; not significant) and with better proliferation rates of unstimulated PBMCs in recovered patients (rs= -0.620; p=0.024). After convalescence, proliferative rates on stimulation with PHA were negatively associated with neopterin (rs= -0.637; p=0.019) and kynurenine (rs= -0.582; p=0.037).

### Discussion

In this study, we have shown a relationship between the status of immune system activation in vivo and in vitro in patients with acute bacterial infections. During acute infection, lower plasma tryptophan as well as elevated neopterin concentrations were observed, which did not reach normal values after convalescence. Higher plasma neopterin values and enhanced IDO activity were associated with impaired proliferative responses of PBMCs upon stimulation with PHA in vitro.

Acutely ill patients had significantly higher plasma neopterin and lower tryptophan concentrations than healthy controls. Higher neopterin was associated with enhanced IDO activity and decreased tryptophan concentrations. Also after convalescence, neopterin

#### Table 1: Median and Quartiles of the parameters of immune system activation. Significant p-values and the related parameters are printed in bold. P value 1 relates to acutely ill patients vs healthy controls, p value 2 relates to convalescents vs healthy controls and p value 3 to acutely ill vs convalescent patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acute Infection</th>
<th>Convalescence</th>
<th>Healthy Controls</th>
<th>p-value 1</th>
<th>p-value 2</th>
<th>p-value 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 24</td>
<td>n = 16</td>
<td>n = 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>7.08 (3.9 - 10.99)</td>
<td>0.41 (0.15 - 1.09)</td>
<td>0.17 (0.1 - 0.44)</td>
<td>0.001</td>
<td>0.112</td>
<td>0.001</td>
</tr>
<tr>
<td>Leukocytes (G/µl)</td>
<td>6.9 (5.5 - 8.4)</td>
<td>7.3 (5.9 - 9.4)</td>
<td>5.9 (5.6 - 7.3)</td>
<td>0.435</td>
<td>0.293</td>
<td>0.826</td>
</tr>
<tr>
<td>Segmentated Neutrophils (%)</td>
<td>69.1 (61.35 - 78.0)</td>
<td>64.7 (58.5 - 70.0)</td>
<td>57.1 (52.13 - 65.35)</td>
<td>0.054</td>
<td>0.186</td>
<td>0.279</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>18.8 (12.25 - 24.6)</td>
<td>23.8 (21.0 - 30.3)</td>
<td>28.95 (26.75 - 35.43)</td>
<td>0.01</td>
<td>0.213</td>
<td>0.033</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>11.7 (10.95 - 12.5)</td>
<td>12.8 (12.25 - 13.95)</td>
<td>13.85 (12.7 - 14.6)</td>
<td>0.019</td>
<td>0.483</td>
<td>0.005</td>
</tr>
<tr>
<td>Iron (µmol/l)</td>
<td>6.5 (4.5 - 10.9)</td>
<td>16.2 (10.0 - 19.6)</td>
<td>16.9 (12.6 - 21.3)</td>
<td>0.005</td>
<td>0.613</td>
<td>0.008</td>
</tr>
<tr>
<td>Transferrin (mg/dl)</td>
<td>187 (160 - 209)</td>
<td>245 (220 - 275.5)</td>
<td>265.5 (230 - 293)</td>
<td>0.004</td>
<td>0.459</td>
<td>0.002</td>
</tr>
<tr>
<td>Transferrin Saturation (%)</td>
<td>14 (9 - 23)</td>
<td>23 (16 - 36.5)</td>
<td>27.5 (21 - 37)</td>
<td>0.031</td>
<td>0.725</td>
<td>0.040</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>157 (76 - 247)</td>
<td>75 (41 - 302.5)</td>
<td>66 (55 - 87)</td>
<td>0.08</td>
<td>0.697</td>
<td>0.003</td>
</tr>
<tr>
<td>Neopterin (nM)</td>
<td>16.32 (7.7 - 20.08)</td>
<td>9.3 (5.48 - 11.94)</td>
<td>5.25 (4.78 - 6.38)</td>
<td>0.002</td>
<td>0.03</td>
<td>0.053</td>
</tr>
<tr>
<td>Tryptophan (µM)</td>
<td>48.6 (35.29 - 62.67)</td>
<td>52.8 (42.65 - 63.95)</td>
<td>68.35 (62.18 - 77.95)</td>
<td>0.011</td>
<td>0.027</td>
<td>0.307</td>
</tr>
<tr>
<td>Kynurenine (µM)</td>
<td>2.11 (1.74 - 2.87)</td>
<td>1.85 (1.36 - 2.59)</td>
<td>2.16 (1.59 - 2.31)</td>
<td>0.451</td>
<td>0.712</td>
<td>0.191</td>
</tr>
<tr>
<td>Kyn/Trp (µM/mM)</td>
<td>48.65 (31.28 - 61.21)</td>
<td>34.39 (27.07 - 47.32)</td>
<td>27.76 (24.18 - 36.15)</td>
<td>0.06</td>
<td>0.21</td>
<td>0.14</td>
</tr>
<tr>
<td>C/N (mg/mM)</td>
<td>4.35 (1.98 - 12.03)</td>
<td>0.4 (0.2 - 1.23)</td>
<td>0.25 (0.0 - 0.9)</td>
<td>0.001</td>
<td>0.499</td>
<td>0.004</td>
</tr>
</tbody>
</table>
values were still increased and tryptophan reduced compared with healthy controls. Elevated neopterin concentrations after convalescence were associated with decreased lymphocyte counts. In a study on patients with acute dengue virus infection, a similar course of tryptophan concentrations were observed, although after convalescence, tryptophan values reached normal levels again [31]. For patients with acute febrile infections other than dengue, in contrast to our study, no differences in tryptophan concentrations during the course of the infection could be found [31]. In the present study, no difference in the course of kynurenine concentrations could be shown. In patients with acute dengue virus infection, increased IDO activity was demonstrated also after convalescence, although this applied only for patients with confirmed dengue infection and not for patients with other febrile infections [31]. Our finding is thus in accordance with the results of a study on patients with acute hanta virus infection, in which IDO levels after

**Figure 1:** Parameters of immune system activation during and after an acute infection compared with healthy controls.

**Figure 2:** Proliferation rates of PBMC upon stimulation with mitogens Con A, PHA and PWM. Significant p-values are mentioned next to the figure.
convalescence were lower than during the infection, albeit higher than the standard values of healthy controls [32]. In the present study, IDO activity, which was calculated via kyn/trp, tended to be lower after convalescence than during an acute infection, but was still higher than in healthy controls.

Both neopterin production and IDO activity are induced by IFN-γ, which is, among other cytokines, mainly produced by Th-1-cells [3,13,33]. Neopterin is used as an indicator of cellular immune activation [34,35], and especially in combination with CRP it can be used as a marker for viral and severe infections [7-9]. In critically ill ICU patients, neopterin measurements could differentiate between a systemic inflammatory response syndrome (SIRS) without infection and sepsis of infectious etiology with a specificity of 78% [8]. Neopterin values higher than 10 nM/L were highly sensitive in the diagnostics of viral respiratory infections, but the specificity was low [36]. Patients with protracted bacterial infections presented with higher neopterin concentrations in their urine than patients with acute infections [37]. A probable reason for this could be the involvement of cellular defense mechanisms in protracted or severe infections [37]. The best diagnostic marker for the differentiation between bacterial and viral respiratory infections turned out to be the ratio of CRP to neopterin [9]. The cut-off value of 3 mg/nM for bacterial infections showed a sensitivity of 93.1% and a specificity of 93.0% [9]. The median ratio for C/N in bacterial infections was 12.5, for viral infections 1.2 and 0.3 mg/nM in healthy controls [9]. In our study with various acute bacterial infections, the mean C/N ratio in acutely ill patients was 4.35 mg/nM, which was significantly higher than in convalescents and healthy controls. Exceptions for the applicability of the C/N ratio are given in patients with renal insufficiency [38] and pediatric patients [39].

Through IDO activity, the availability of tryptophan in the surrounding microenvironment is limited and thus deprived from protein synthesis and proliferation [15]. This mechanism provides an antiproliferative action against pathogens and tumor cells [26-29]. The immunoregulatory effects of IDO have been described based on the experiments of Munn et al. [20]. Tryptophan degradation via IDO is necessary to ensure fetal tolerance by the mother’s immune system. After inhibition of IDO with 1-Methyltryptophan (1-MT), pregnancy was not possible. [20] T-cells were found to react sensitively to tryptophan shortage and persist in G1-phase of the cell cycle [40]. After a latency period of 12-16 hours, the entry into S-phase could be achieved by resupplementation of tryptophan, which indicates reversibility of the inhibition of proliferation [41]. Likewise, kynurenine and picolinic acid were shown to have antiproliferative effects on T-cells [42,43]. In contrast to inhibition via tryptophan depletion, T-cells could not be restimulated after inhibition through kynurenine [42,43]. On the basis of time and dose dependent cytotoxicity and the longer exposition in vivo compared to in vitro conditions, even small concentrations of kynurenine could have toxic effects on activated T-cells [42].

Antiparasitic activity was first described against the intracellular pathogen Toxoplasma gondii [28]. IDO activity was shown to have antibacterial and antiviral effects against a variety of intracellular and extracellular pathogens [7]. As an explanation for these contrary functions of IDO, on the one hand inhibiting pathogen growth and on the other hand arresting T-cells, different minimum concentrations of tryptophan for bacterial and T-cell growth were proposed [27]. Accordingly, bacteria require a tryptophan concentration 10 to 40 times higher than T-cells to proliferate [27]. During an ongoing immune reaction, tryptophan depletion would thus firstly cause
an inhibition of bacterial growth and later T-cell arrest, which then autoregulates the immune reaction through decreased production of IFN-γ [27]. Kynurenine seems to have impact only on T-cells and not on bacterial proliferation [27].

Elevated IDO activity and neopterin concentrations have been found in HIV infected individuals, especially in disease progression [18,44,45]. A vicious cycle of immune activation-induced immunosuppression and thus increased susceptibility to infection with further immune activation seems to represent the optimal conditions for HIV replication [18]. As a result, neopterin represents a sensitive prognostic marker for disease progression in HIV-1 and HIV-2 infections [46,47]. Abnormal kyn/trp rates have been associated with earlier occurrence of AIDS-defining illnesses [48]. Systemic immune activation thus seems to accelerate disease progression and impair survival for HIV infected individuals [49-51]. Effective antiretroviral therapy (ART) hence slows down disease progression not only via inhibition of virus replication, but also through reduction of IDO activity [44,52]. During the course of the disease, immune cells appear to reach a state of “exhaustion” [18], which is not yet fully explained. Exhaustion is defined as a loss of the defensive function and the proliferative capacity [53]. Depending on disease duration, T-cells of HIV infected patients show reduced proliferative responsiveness to stimulation compared with healthy controls [54,55], and stimulated T-cells of patients with HIV produce less IFN-γ [56].

In our study, older patients tended to present with higher neopterin concentrations than younger individuals. Patients above the age of 65 with acute infections showed significantly lower tryptophan values, and older age was significantly associated with enhanced IDO activity. This is in line with the results of other studies concerning increased immune activation with older age [57-59]. To which extent this increase in cellular immune activation constitutes a condition of normal aging or rather results of more frequently occurring cell mediated pathologies in the elderly, remains unclear [60].

In this study, unstimulated PBMCs of acutely ill patients proliferated significantly better than those of convalescents. Also upon stimulation with Con A, the highest proliferation rate was reached during acute infection. Stimulated with Con A as well as PHA, the cells of younger patients’ proliferated better than those of older patients. There was an inverse relationship between plasma neopterin and proliferation upon PHA stimulation during as well as after acute infection in vitro, and a positive correlation between proliferative rate and tryptophan concentration existed. Also, after convalescence, high kynurenine and kyn/trp were associated with decreased proliferative responses on PHA. These results could represent the immune system’s normal reaction to an acute infection. During an acute infection, the increase in immune system activation parameters and higher proliferation rates of immune cells would have positive effects on pathogen control. Already during the combat phase, IDO mediated tryptophan depletion and decrease of cytokine producing cells would dampen the proliferation rate of immune cells and likewise the immune reaction. Since the patients in this study did not present with chronic virus infections in connection with persistent immune activation, this downregulation would work efficiently and not lead to a deregulation with subsequent exhaustion of the immune system.

In experiments with PBMCs of HIV infected patients, decreased proliferation rates were observed compared to healthy controls [30]. Furthermore, negative correlations between neopterin concentration and proliferative rates on stimulation with PHA and PWM were found [30]. Also, other studies have focused on the responsiveness of immune cells in HIV infection [54,61], however due to its chronicity HIV infection constitutes an immunological situation that is not comparable to acute infections as in the present study. In other studies about acute viral infections, a transient suppression of PBMC proliferation has been documented. After complete convalescence, proliferative responsiveness to stimulation was on a normal level again [62-64]. In none of those studies with acute infections, however, in vitro proliferative responses have been evaluated for associations with immune system activation parameters in vivo during and after acute infections of different etiology. The present study could therefore provide insight into the complex connections between immune system activation and proliferation of immune cells as well as the feedback mechanisms in the course of convalescence. It is certainly true, that our experimental model system employing PBMCs does not allow us to draw firm conclusions about the role of different cell types within the immune response –we also did not measure percentages of NK cells, NKT cells or B cells. These percentages may also vary depending on the situation and also the underlying disease of the patient. We chose our experimental setting because in our earlier experiments we found that PBMC experiments are suited well to assess the immune response as a whole and because production of IFN-γ correlates well with the formation of neopterin and the degradation of tryptophan in vitro as well as in vitro in HIV-infected patients [65].

In this study we did not determine levels of cytokines like IFN-γ, type I interferons or Interleukin 2, as the half-
life of cytokines varies greatly and it is a challenge to find the right time to measure several cytokines - especially when patients suffer from other underlying bacterial diseases and also have different comorbidities.

Instead we measured neopterin and tryptophan degradation, because it has been proven to be robust and correlates with the course of disease in vivo as well as in vitro. Thus, we assume that it might be more meaningful than the determination of cytokine levels. In line with this hypothesis, PBMC experiments investigating neopterin formation and tryptophan degradation have also been proposed as valuable tests to investigate the capacity of immunomodulatory compounds [66].

Still, further studies with larger and more homogenous study populations might provide additional information about the complex counter regulations in different immunological situations.

Conflict of interest: Authors state no conflict of interest

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