Glutamine-Enriched Enteral Nutrition Increases HLA-DR Expression on Monocytes of Trauma Patients1,2


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ABSTRACT The aim of this study was to investigate the effect of glutamine-(Gln)-enriched enteral nutrition (EN) on human leukocyte antigen (HLA)-DR and FcγRI/CD64 expression on monocytes and plasma glutamine concentrations in multi-trauma patients. HLA-DR expression on monocytes is crucial in the presentation of foreign antigen to the immune system and is severely reduced in trauma patients. In vitro monocyte HLA-DR and FcγRI/CD64 expression is dependent on glutamine availability. To study the effect of glutamine supplemented enteral nutrition on HLA-DR and FcγRI/CD64 expression on CD14+ monocytes, 55 multi-trauma patients were studied in a randomized, double-blinded, controlled trial. Trauma patients received either a Gln-enriched EN (glutamine group, n = 28) or an isocaloric, isonitrogenous control EN (control group, n = 27) and were compared with a group of age-matched healthy volunteers (healthy volunteers, n = 53). On d 1, 5, 9 and 14 after trauma, expressions of HLA-DR and FcγRI/CD64 were determined on CD14+ monocytes using FACS analysis. Plasma glutamine levels were measured using HPLC. Plasma glutamine was lower in both trauma patient groups compared with healthy volunteers and from d 3 to d 5; glutamine was higher in the glutamine group than in the control group. On d 1, HLA-DR expression was much lower in both trauma patient groups than in healthy volunteers. HLA-DR expression was greater on d 5, 9 and 14 in the glutamine group than in the control group. FcγRI/CD64 expression on monocytes of trauma patients was not different than the expression of healthy volunteers. This study showed that glutamine-enriched enteral nutrition was associated with a higher HLA-DR expression on CD14+ monocytes of trauma patients. No difference in monocyte FcγRI/CD64 expression was detected between patients that received the two enteral diets and between trauma patients and the healthy volunteers. Increased HLA-DR expression may improve cellular immune function and may be involved in the beneficial effect of glutamine on the occurrence of infections in trauma patients. J. Nutr. 132: 2580–2586, 2002.

KEY WORDS: • glutamine • human leukocyte antigen DR • Fc receptor • trauma • surgery

Infectious complications remain a serious problem after critical injury, which may be related to disturbed cellular immune function (1–4). This immune state is characterized by a depressed expression of the major histocompatibility (MHC)4 class II antigens (HLA-DR) on monocytes, which play a critical role in the induction of the cellular immune response to foreign antigen (5). T lymphocytes only recognize extracellular bacterial antigens when HLA-DR molecules are expressed in close proximity to the antigen on the presenting monocyte or macrophage (6). In trauma patients, the expression of HLA-DR antigens on monocytes is severely depressed, the magnitude of which correlates with clinical outcome (1,7,8). Maintenance of HLA-DR expression, therefore, may be pivotal in mounting an adequate antibacterial immune response and in preventing infectious complications in trauma patients. In addition to HLA-DR expression, another important surface marker in the monocyte-dependent immune responses is the FcγRI/CD64 receptor that triggers activity upon binding of the subclass immunoglobulin G (IgG). (9) This monocyte receptor links humoral and cellular immunity by serving as a bridge between antibody specificity and effector cell function (10).

Recently, it has been shown that the in vitro expressions of HLA-DR antigen and FcγRI/CD64 on blood monocyte-derived macrophages were dependent on the availability of glutamine in the medium (11). In trauma patients, we and others

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have shown that plasma levels of glutamine are reduced up to 40% or more (12,13), which may underlie the reduced HLA-DR expression in these patients (1,14).

In this study, we investigated the effect of a glutamine-supplemented enteral nutrition on the expression of HLA-DR and FcγRI/CD64 on monocytes in multi-trauma patients during the first 15 d of their hospital stay.

PATIENTS AND METHODS

This study, which is part of a larger study on the effects of enteral glutamine supplementation in trauma patients, was approved by the Council for Medical Research of The Netherlands Organization for Scientific Research (900-716-064) (12) and by the Institutional Review Board of our institute. Informed consent was obtained from each patient or the closest relative before inclusion.

Patients. Fifty-five multi-system trauma patients admitted to the intensive care unit of the VU University Medical Center (Amsterdam, The Netherlands) and the Gelsemum Ziekenhuis Hospital (Nijmegen, The Netherlands) were enrolled eligible for this study. Each patient with a multiple trauma was assessed by the Injury Severity Score (ISS), the Acute Physiology and Chronic Health Evaluation (APACHE) II score, and the Glasgow Coma Scale. Patients between the ages of 18 and 65 y, with an expected survival of >48 h, an ISS of ≥20 (15), and at least 5 d of enteral nutrition were included.

The exclusion criteria were pregnancy or lactation; renal insufficiency; third degree burns involving >15% body surface; use of other investigational drugs, steroids, or immunosuppressive medication; malignancy; genetic disorder; human immunodeficiency virus (HIV) infection; splenectomy before hospitalization; liver insufficiency; inflammatory bowel disease and insulin-dependent diabetes.

Infectious morbidity was scored during the first 15 d by criteria published previously (12). The aim of this study was to investigate the effect of enteral glutamine-enriched nutrition on HLA-DR and FcγRI/CD64 expression of CD14+ monocytes of trauma patients.

Patients were randomly allocated to glutamine-supplemented enteral nutrition or balanced isonitrogenous enteral feeding. After study application, forms were signed by the patient’s physician and the pharmacist used a computer-generated randomization table based on blocks of four to assign patients to treatment A or B, which corresponded to batch numbers on the nutrition pouches. The code for the batch numbers was kept at the supplier and the pharmacist used a computer-generated randomization table based on blocks of four to assign patients to treatment A or B, which corresponded to batch numbers on the nutrition pouches. The code for the batch numbers was kept at the supplier’s office in the United States and the codes were broken after the analyses were finished. All participants were unaware of treatment allocation.

The enteral nutrition was freshly prepared in the pharmacy department by mixing the dry powder with sterile water and was then placed in brown glass bottles labeled with the patient’s name, hospital number and protocol number. To ensure the double-blind design of the study, the solutions were indistinguishable by color, smell or taste.

Study design. The evaluable patients were randomly assigned to receive either a glutamine-supplemented enteral nutritional formula (30.5 g/100 g protein, AtrilAQ; Ross Products Division, Abbott Laboratories, Columbus, OH; n = 28, Qln) or a balanced enteral control solution (n = 27, controls, Table 1) and were compared with age-matched healthy volunteers (Table 2).

The control solution was identical to the glutamine-enriched solution except that the following amino acids were added in place of glutamine: alanine, aspartic acid, glycine, proline and serine. After immediate stabilization and surgical treatment, feeding was started within 48 h of admission via a nasoduodenal tube that was inserted endoscopically. Feeding was delivered continuously via a pump with the goal to reach 75% of the calculated basal energy expenditure (BEE) within 72 h of admission (16) (Table 2). The enteral nutrition was continued until the patients tolerated oral feeding. While receiving the experimental nutrition, the patients did not receive any other form of nutrition. A minimum period of 5 d of feeding was set arbitrarily for glutamine to have an effect on HLA-DR and FcγRI/CD64 expression.

Glutamine analysis. Glutamine plasma levels were determined on d 1, 2, 3, 4, 5, 7 and 14. Blood samples were obtained between 0800 and 1000 h, processed, and analyzed by HPLC as previously described (17). The normal range of plasma glutamine was determined to be 482–938 μmol/L (mean: 681 μmol/L; mean age of volunteers: 38 y; range: 19–77 y; n = 44) (17).

Monocyte HLA-DR and FcγRI/CD64 expression. On d 1, 5, 9 and 14, HLA-DR antigen and FcγRI/CD64 expression were measured in fresh heparinized venous blood after lysis of erythrocytes (Q prep; Coulter Corporation, Miami, FL). The absolute numbers of leukocytes and the percentage of lymphocytes and monocytes (CD14+) were determined. The expression of HLA-DR antigen and FcγRI/CD64 on CD14+ cells were evaluated by FACS analysis (FACStar Plus; Beckton Dickinson, San Jose, CA) and were expressed as mean channel fluorescence intensity (MFI). To evaluate the HLA-DR expression, CD14+ cells without HLA-DR, monoclonal antibody (Mab)
were used to adjust the FACStar Plus, after which the MCF of the HLA-DR-positive cells within the CD14 gate was established. The FcyRI/CD64 expression was obtained similarly. The Mab (anti HLA-DR-FITC, anti-FcγRI/CD64-FITC and anti-CD14) receptors were purchased from Becton Dickinson (San Jose, CA). Healthy age-matched volunteers were used for comparison of the immunological assessment.

**Statistical analysis.** To compare between two patient groups at the moment of study entry, Student’s t test was used to compare age and BEE and a Mann-Whitney U test was used for the clinical assessment scores. The distribution of sites of injury and types of surgery as well as the appearance of infections were analyzed by means of a χ² test. Differences in plasma glutamine levels, CD14, CD64, HLA-DR expression between the glutamine-treated and control patients and healthy volunteers were analyzed using the two-factor repeated-measures ANOVA with interaction. Significances of the differences at each time point were analyzed using the Student’s t test using Bonferroni’s correction. The analysis was done using a commercial statistical package (Stat-View; Abacus Concepts, Berkeley, CA) on a Macintosh computer (Apple Computer, Cupertino, CA). Results are given in means ± SEM. Differences with P values < 0.05 were considered significant.

### RESULTS

A total of 80 multiple trauma patients were randomly assigned to receive glutamine-enriched enteral nutrition (Gln, n = 41) or isonitrogenous, iso-caloric control nutrition (control, n = 39). Three patients were ineligible because of age violation (n = 2) or as a result of established HIV infection (all Gln). Five patients dropped out of the study for the following reasons: death within 48 h (2 Gln and 1 control) or transfer to another hospital within 48 h (1 Gln and 1 control). Another 12 patients were fed enterally for fewer than 5 d for several reasons (6 Gln and 6 controls). The results presented in this study were obtained from the two study groups of patients who were fed for at least 5 d enterally (28 Gln vs. 27 controls) and were compared with age-matched healthy volunteers.

**Clinical monitoring.** At hospital admission there were no differences in baseline characteristics (Table 2). Nutritional intakes did not differ between the two patient groups (results not shown) and there was no difference in the number of days of enteral nutrition (Gln, 12.8 ± 1.1 d; control, 11.9 ± 1.0 d). There were no differences in the type of injury or surgical intervention (Table 3).

**Infectious morbidity.** Patients receiving the glutamine-enriched feeding had a significantly lower incidence of pneumonia (P < 0.05), bacteremia (P < 0.005) and sepsis (P < 0.05; Table 4). No differences were seen for other types of clinical infections (data not shown).

**Glutamine.** There was evident glutamine depletion in both groups of trauma patients as shown by the low plasma glutamine levels measured at d 1 (Fig. 1). The reference range of glutamine was 482–938 μmol/L with a mean of 681 ± 13.32 μmol/L (17). Plasma glutamine levels were greater in trauma patients receiving glutamine-enriched nutrition than in the control group on d 3 (P < 0.005), 4 (P < 0.005) and 5 (P < 0.05). In the control group, glutamine levels also increased, resulting in similar plasma levels from d 7 onward in the two patient groups.

**Antigen expression on monocytes.** HLA-DR expression was very low on monocytes taken from both patient groups at d 1 (MCF): Gln, 51.0 ± 11.7 (n = 28); controls, 49.2 ± 5.2 (n = 27); and healthy volunteers, 191.9 ± 19.1 (n = 53) (Fig. 2). In the control group, the HLA-DR expression declined further on d 5, which did not occur in the glutamine group. In

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**Table 2**

Baseline characteristics of trauma patients receiving glutamine-enriched enteral nutrition (EN) or an isonitrogenous control EN and healthy volunteers

<table>
<thead>
<tr>
<th></th>
<th>Glutamine</th>
<th>Control</th>
<th>Healthy volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>28 (53)</td>
<td>27 (53)</td>
<td></td>
</tr>
<tr>
<td>Male, n</td>
<td>25 (53)</td>
<td>20 (45)</td>
<td></td>
</tr>
<tr>
<td>Female, n</td>
<td>3 (6)</td>
<td>4 (8)</td>
<td></td>
</tr>
<tr>
<td>Age, y (range)</td>
<td>25.2 (22–55)</td>
<td>34.6 (19–59)</td>
<td>31.2 (22–52)</td>
</tr>
<tr>
<td>ISS</td>
<td>32.8 ± 2.3</td>
<td>31.8 ± 1.7</td>
<td>0</td>
</tr>
<tr>
<td>APACHE II</td>
<td>15.8 ± 1.1</td>
<td>15.9 ± 1.0</td>
<td>0</td>
</tr>
<tr>
<td>GCS</td>
<td>8.6 ± 0.9</td>
<td>8.4 ± 0.6</td>
<td>15</td>
</tr>
<tr>
<td>BEE, kcal/d</td>
<td>2620.1 ± 68.7</td>
<td>2580.1 ± 78.3</td>
<td>n.d.</td>
</tr>
<tr>
<td>BEE, MJ/d</td>
<td>10.96 ± 0.29</td>
<td>10.80 ± 0.33</td>
<td></td>
</tr>
<tr>
<td>Energy intake, MJ/d</td>
<td>11.00 ± 0.29</td>
<td>10.84 ± 0.33</td>
<td></td>
</tr>
</tbody>
</table>

1 Each patient was clinically assessed by the Injury Severity Score (ISS), the Acute Physiology and Chronic Health Evaluation (APACHE) II score, and the Glasgow Coma Scale (GCS). Furthermore, the Basal Energy Expenditure (BEE) was determined. Data are expressed as means ± SEM. Groups did not differ as indicated by Student’s t test or Mann-Whitney U test.

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**Table 3**

Distribution of type of injury and type of surgery of trauma patients receiving glutamine-enriched enteral nutrition (EN) or the isonitrogenous control EN

<table>
<thead>
<tr>
<th></th>
<th>Glutamine (n = 28)</th>
<th>Control (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of injury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thorax</td>
<td>10 (11)</td>
<td>11</td>
</tr>
<tr>
<td>Abdomen</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Thorax and abdomen</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Extremities</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Head injury</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laparotomy</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Thoracotomy</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Neurosurgery</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Operative fracture treatment</td>
<td>9</td>
<td>8</td>
</tr>
</tbody>
</table>

1 Data were analyzed by a χ² test; groups did not differ.

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**Table 4**

Frequency of infections in trauma patients fed for more than 5 d a glutamine-enriched enteral nutrition (EN) solution or an isonitrogenous control EN

<table>
<thead>
<tr>
<th></th>
<th>Glutamine (n = 28)</th>
<th>Control (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia</td>
<td>5 (18)</td>
<td>14 (52)</td>
</tr>
<tr>
<td>Bacteremia</td>
<td>2 (7)</td>
<td>12 (44)</td>
</tr>
<tr>
<td>Sepsis</td>
<td>1 (4)</td>
<td>8 (30)</td>
</tr>
</tbody>
</table>

1 Values are numbers of subjects, with the percentage of patients with the diagnosis per study group in the parentheses, n = 55.

2 Different from the control group at *P < 0.05; **P < 0.005; χ² test.
the glutamine group, higher HLA-DR expression was found on monocytes taken on d 5 (P < 0.05), d 9 (P < 0.05) and d 14 (P < 0.05) compared with the expression at d 1. Representative plots are shown in Fig. 3. FcγRI/CD64 expression did not differ between healthy volunteers (MCF; 153.4 ± 17.7, n = 23) and both trauma patient groups, which also did not differ. The leukocyte counts did not differ between the trauma patients on any of the days under investigation (data not shown). The percentage of CD14+ cells in whole blood was higher (P < 0.001) in both the glutamine and control groups on d 1 and d 5 compared with the volunteers. On d 9, the percentage was greater in the control group only (P < 0.05) (Fig. 4). On d 14, the percentages of CD14-positive cells of trauma patients were not different from those of healthy volunteers. At no time point did the glutamine and control groups differ in percentage CD14+ cells (Fig. 4).

**DISCUSSION**

Trauma patients have an early-onset depression of the overall cellular immune response (3,18), which is associated with a high rate of infections and considerable mortality (1,2,4). In terms of surface recognition, monocytes play a crucial role in the cellular immune response because they function both as antigen-presenting cells, dependent on HLA-DR expression, and as effector cells, when triggered by binding of IgG to FcγRI/CD64 receptors (5,6). In trauma patients, Polk et al. (7,8,19) have shown that the expression of monocyte HLA-DR is severely reduced and correlates with outcome. The dependency of HLA-DR expression on glutamine has been reported by Spittler et al. (11). In their in vitro experiments they found that when cultured for 1 wk in the presence of reduced concentrations of glutamine, blood monocyte-derived macrophages express progressively fewer HLA-DR antigens on their surfaces. In trauma patients, plasma glutamine levels are severely reduced and like in this study (Fig. 1) may be 40% or more lower than in healthy volunteers [mean: 681.0 (482–938) μmol/L; n = 44] (11,17,13). At these glutamine concentrations (400 μmol/L), Spittler et al. (11) found a 30% reduction in HLA-DR and a 15% lower FcγRI/CD64 expression in vitro.

In an attempt to increase HLA-DR expression on monocytes in humans the effect of glutamine-enriched enteral nutrition on monocyte HLA-DR antigen and FcγRI/CD64 expression was studied in a double blind randomized trial. This approach produced groups comparable in terms of age, sex, type and severity of injury and importantly the number of days of enteral nutrition and mean daily energy intake (Table 2).

The very low expression of HLA-DR antigen on monocytes of trauma patients at the 1st d after trauma confirms previous results in trauma patients (Fig. 2) (8). The key finding of this study was that the glutamine-enriched enteral nutrition increased the expression of HLA-DR on monocytes in the first
15 d, a period in which most infections develop (Fig. 2) (20). This greater expression of HLA-DR coincided with significantly higher plasma glutamine levels in glutamine-supplemented patients within the 1st wk. The latter suggests improved glutamine availability in supplemented patients (Fig. 1). We, therefore, conclude that compared with a balanced control feeding, the glutamine-enriched enteral nutrition increased HLA-DR expression on monocytes of trauma patients. These results of increased HLA-DR expression on monocytes of trauma patients receiving enteral glutamine administration provide for the first time an in vivo manifestation of the in vitro results of increasing HLA-DR expression by adding glutamine to a culture medium. The enteral route of administering nutrition was previously described by Kudsk et al. (20) to be preferred particularly in severely injured patients. Recently it was also shown by Spittler et al. (21) that a parenteral supplementation with the dipeptide glycyI-glutamine partially prevented HLA-DR decrease in abdominal surgical patients in the first 48 h postoperatively. This means that both routes of administration of glutamine, enterally or parenterally, could augment/ameliorate the expression of HLA-DR on CD14-positive monocytes.

In contrast to the HLA-DR antigen, the expression of the FcγRI/CD64 receptor on monocytes was not reduced in trauma patients in this study. This was unexpected because similar to the HLA-DR expression, the in vitro expression of FcγRI/CD64 receptors also depended upon the medium glutamine concentration in the experiments of Spittler et al. (11). Our results, therefore, suggest that the expression of FcγRI/CD64 receptors on monocytes of trauma patients is not influenced by glutamine. We also conclude that the IgG-mediated monocyte response in trauma patients will not be disturbed by low glutamine availability. Therefore, it seems that monocyte dysfunction in trauma patients may be explained at least in part by disturbed HLA-DR antigen presentation but most likely not by a diminished number of receptor sites for IgG antibody-dependent immune responses.

Recently, our group has shown that glutamine-enriched enteral nutrition reduces the incidence of pneumonia, sepsis and bacteremia in critically injured patients (Table 4) (12). Houdijk et al. (12) also reported lower levels of soluble TNF receptors in the glutamine-enriched group, suggesting a modulated systemic inflammatory response. Huang et al. (23) and Ertel et al. (22) showed a positive correlation between serum sTNF receptor levels and activities of infectious and inflammatory diseases. In line with a positive effect of glutamine on immune function, the increase in HLA-DR expression on monocytes of patients fed the glutamine-enriched diet may also contribute to a more adequate inflammatory response. In parallel with the effect of glutamine on HLA-DR expression, the in vitro phagocytic capacity of blood monocyte-derived macrophages is also dependent on the amount of glutamine in the culture medium (11). Furthermore, in favor of an antibacterial effect of glutamine, it recently was shown that glutamine-enriched enteral diets increase the bacterial clearance of intraperitoneally delivered Escherichia coli in rats (24).

Fourteen days after the trauma, the expression of HLA-DR on monocytes was still much lower than in normal controls (Fig. 2), which confirmed the results of Hershman et al. (8) and Furukawa et al. (24). The increase in HLA-DR expression in the glutamine-enriched group of trauma patients did not restore normal values. This was also seen for the plasma

**FIGURE 2** HLA-DR antigen expression on CD14+ monocytes of trauma patients receiving glutamine-enriched enteral nutrition (EN) (n = 28) and control feeding (n = 27) compared with healthy volunteers (hatched area, n = 53). All results are expressed as mean channel fluorescence (MCF) ± SEM. Significant differences between the two patient groups are indicated by *P < 0.05.
FIGURE 3 Profiles of the HLA-DR expression on CD14+ monocytes of one patient fed the glutamine-enriched solution (A) and one patient fed the isocaloric and isonitrogenous control solution (B) on d 1, 5 and 9 (left, middle and right panels, respectively), as well as one healthy volunteer (C). Gated populations of HLA-DR-positive (upper panel) and -negative (lower panels) CD14+ monocytes are shown. To evaluate the HLA-DR expression, CD14 + cells without HLA-DR MAb were used to adjust the FACStar Plus, after which the mean channel fluorescence (MCF) of the HLA-DR-positive cells within the CD14 gate was established.
glutamine levels because these only reached the lower range of the determined reference levels even after 14 d of glutamine-enriched feeding (Fig. 1). The extreme loss of HLA-DR expression early after trauma (75%) was more than the 30% decrease Spittler et al. (11) found based on the plasma glutamine concentration found in this study. The loss of HLA-DR expression, therefore, is most likely not explained by lower glutamine availability. Since the recovery of HLA-DR monocytic expression was not completely restored by glutamine supplementation, it would be of interest to study the effect of the administration of higher amounts of dietary glutamine. This may be achieved by using more enteral supplements or by using a combined strategy of both enteral and parenteral glutamine-supplemented nutrition.

LITERATURE CITED


