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# Plasma glutamate levels, lymphocyte reactivity and death rate in patients with bronchial carcinoma

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**Summary.** Elevated glutamate concentrations are commonly observed in patients with advanced carcinoma, and glutamate was recently found to inhibit the membrane transport of cystine and to impair the function of macrophages and lymphocytes in vitro. We therefore investigated the possibility that elevated plasma glutamate levels may be quantitatively correlated with reduced lymphocyte reactivity and an impaired host response to the tumor. Here we report the results of a study on patients with bronchial carcinoma, which show that patients with plasma glutamate levels above 120 $\mu M$ have a lower lymphocyte response to mitogens and a substantially higher death rate than those with glutamate levels below 120 $\mu M$. This correlation does not prove a causal role of glutamate, but it confirms predictions from the in vitro laboratory data.

**Key words:** Plasma glutamate levels – Lymphocyte reactivity – Death rate – Bronchial carcinoma

## Introduction

Glutamate has been shown to inhibit competitively the membrane transport of cystine (Watanabe and Bannai 1987). Presumably as a consequence of this effect, elevated extracellular glutamate concentrations reduce the intracellular cysteine concentration in macrophages and the capacity of these cells to release cysteine into the extracellular space (Eck and Dröge 1989). Although the consequence of the reduced cysteine supply for the macrophage itself has not yet been studied in detail, there is increasing evidence that the capacity to release cysteine may be important for T cells. It has been shown that the activation of cytotoxic T lymphocytes (CTL) and the DNA synthesis of (a subset of) T cells and T-cell clones is strongly dependent on intracellular glutathione levels and stronlgy influenced by variations of the extracellular cysteine concentration, even in the presence of several-fold higher concentrations of cystine and methionine (H. Gmünder et al., submitted for publication; see also Eck et al. 1989). The blood plasma concentration of cysteine (10–20 $\mu M$) is exceptionally low in comparison to that of other amino acids (Eck et al. 1989; Saetre and Rabenstein 1978; Chawla et al. 1984); T cells are therefore expected to depend on the elevated concentrations of cysteine in the vicinity of macrophages, since these cells are capable of taking up cystine (plasma concentration, 100–200 $\mu M$ 1/2 cystine) and of releasing substantial quantities of cysteine into the extracellular space (Eck and Dröge 1989). It is well established that macrophage-like cells play an important role as stimulator and/or accessory cells in T-cell responses and come into intimate contact with T cells (Schwab et al. 1985; Rosenstreich et al. 1976; De Vries et al. 1979; Thiele and Lipsky 1982; Rosenthal 1978).

Taken together, these laboratory experiments lead to the prediction that patients with pathologically elevated plasma glutamate concentrations may have impaired lymphocyte functions and secondary immunopathological consequences. A disease that results in a most striking elevation in plasma glutamate levels is the acquired immunodeficiency syndrome (AIDS) (Eck et al. 1989; Dröge et al. 1988a). Elevated glutamate levels have been found in HIV-antibody-positive persons without symptoms and even in a proportion of persons without HIV-1 antibodies who were considered to be at risk of developing AIDS (Dröge et al. 1988a). This indicates that elevated glutamate levels may be among the first consequences of HIV-1 infection and may even precede seroconversion. HIV-antibody-positive persons have also been shown to have abnormal lymphocyte functions before their T-cell counts were substantially reduced (Miedema et al.

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1988). AIDS and pre-AIDS conditions therefore provide an obvious example of a strong coincidence of elevated glutamate levels and reduced lymphocyte functions. However, since there is no therapeutic strategy available to reduce the glutamate levels in these patients, there is presently no possibility to prove directly the pathogenetic role of glutamate in this disease.

Another disease associated with elevated plasma glutamate levels is cancer. Elevated glutamate levels have been reported for patients with various types of advanced cancer (reviewed in Dröge et al. 1988 b); it was therefore of interest to test the prediction derived from the laboratory data that the elevated glutamate levels may also be correlated in these cases with reduced lymphocyte functions and possibly, with a reduced host response to the tumor. We report the results of a study on patients with bronchial carcinoma, which show that patients with high glutamate levels (i.e. $>120$ $\mu M$) have indeed, on the average, markedly lower lymphocyte reactivity and a higher death rate per year than those with glutamate levels below 120 $\mu M$.

## Patients and methods

*Patients.* The study originally included 40 patients with small-cell carcinoma and 24 patients with non-small-cell carcinoma of the lung who were admitted to the Hospital for Thorax Diseases in Heidelberg-Rohrbach. Four of the patients with small-cell carcinoma and three of those with non-small-cell carcinoma were later excluded from the study because there was no information available as to whether they were still alive at the end of the study, 1.5 years after their admission to the hospital. According to current medical practice, all patients received systemic polychemotherapy and/or radiotherapy, depending on the tumor stage.

*Blood samples.* Heparinized (50 units/ml) blood (usually 10 ml) was collected in polysterene tubes (Greiner, Nürtingen). Blood from randomly selected healthy persons over 39 years old (both sexes) was obtained from the Central Blood Service, University of Heidelberg. Blood samples from patients with bronchial carcinoma were taken at the Hospital for Thorax Diseases at Heidelberg-Rohrbach before treatment or surgical operation.

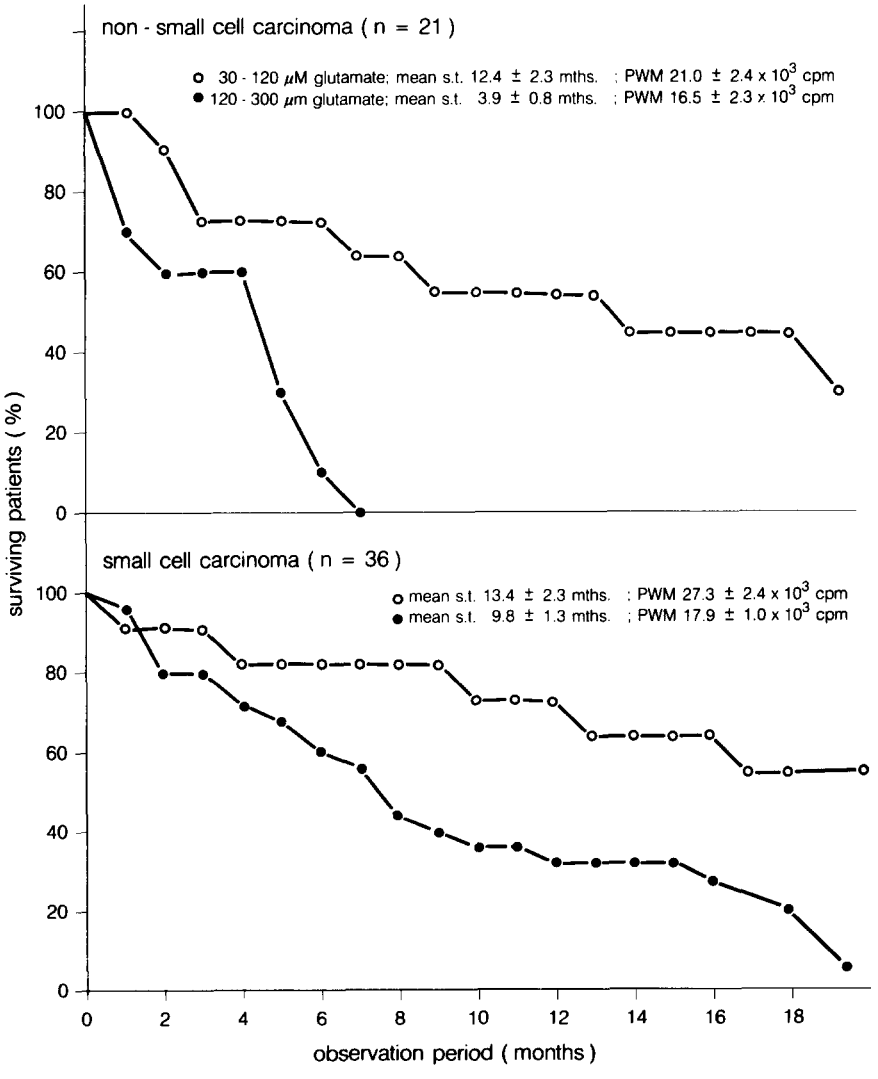


**Fig. 1.** Correlation between plasma glutamate concentration, lymphocyte reactivity and survival in patients with bronchial carcinoma, showing the percentage of survival at different intervals, the mean survival $\pm$SEM and the mitogenic responses against PWM as time zero of patients with 30–120 $\mu M$ plasma glutamate (*open circles*) and those with 120–300 $\mu M$ glutamate (*closed circles*)

*Lymphocyte reactivity in response to pokeweed mitogen.* The mitogenic responses were determined using a whole-blood proliferation assay (Leroux et al. 1985). Briefly, the blood was mixed within 2–3 h after collection at a ratio of 1:15 with RPMI 1640 medium supplemented with 2 m*M* glutamine, 100 IU/ml penicillin, and 100 µg/ml stretomycin but without additional serum. Aliquots of 0.9 ml blood suspension were distributed into microtiter plates (48 wells/plate), and pokeweed mitogen (PWM) was added in aliquots of 0.1 ml (final concentration, 10 µg/ml). After 7 days of incubation, freshly suspended aliquots (200 µl) from each well were transferred into 96-well microtiter plates and incubated for another 4 h with 1 µCi [$^3$H]-thymidine. Thymidine incorporation was determined by a standard procedure using a Dunn cell harvester and a Beckman scintillation counter.

*Amino acid analysis (determination of glutamate).* Plasma samples were mixed with 10% sulfosalicyclic acid (0.126 ml/0.5 ml plasma) and kept for 30 min at 4° C with occasional stirring. The precipitate was removed by high-speed centrifugation and the resulting supernatant was stored at −20° C and finally subjected to amino acid analysis (Biotronic amino acid analyzer LC 2000).

## Results

The lymphocyte reactivity against PWM and the plasma glutamate levels were determined in blood samples that were taken after the patients admission to the hospital before treatment or surgical operation. The sampling of the blood defines time 0 of the observation period (Fig. 1). In all, 25 of the 36 patients (69%) with small-cell carcinoma and 10 of the 21 patients (48%) with non-small-cell carcinoma were found to have a plasma glutamate level of $>120$ µ*M*. All groups of patients showed not only elevated glutamate levels but also substantially reduced mitogenic responses when compared with a group of 37 healthy blood donors (Table 1). However, in both groups of lung cancer patients we found that patients with glutamate levels of $<120$ µ*M* had still higher mitogenic responses than those with glutamate levels of $>120$ µ*M* (Table 1). The difference between the mean mitogenic response of the entire group of 22 patients with glutamate levels below 120 µ*M* and that of the total group of 35 tumor patients with glutamate levels above 120 µ*M* was highly significant according to the Wilcoxon rank-sum test ($P<0.0001$) (Table 1). These results demonstrate that high glutamate levels are associated with low lymphocyte reactivity and that this difference is detectable even when lymphocytes are incubated in a standard cell-culture medium in vitro.

**Table 1.** Mean plasma glutamate levels and lymphocyte reactvity in tumor patients and healthy blood donors

| | Mean plasma glutamate level (µmol/l) | Mitogenic response against PWM (cpm × $10^{-3}$) |
|---|---|---|
| Healthy blood donors | 55.3 | 41.5±3.3 |
| Non-small-cell carcinoma: | | |
| glutamate $<120$ µ*M* | 93.6 | 21.0±2.4 |
| glutamate $>120$ µ*M* | 138.2 | 16.5±2.3 |
| Small-cell carcinoma: | | |
| glutamate $<120$ µ*M* | 82.6 | 27.3±2.4 |
| glutamate $>120$ µ*M* | 161.9 | 17.9±1.0 |
| Att tumors patients: | | |
| glutamate $<120$ µ*M* | | 23.9±1.8[a] |
| glutamate $>120$ µ*M* | | 16.8±1.0[a] |

[a] $P<0.0001$ according to the Wilcoxon rank-sum test

This suggested the possibility that the elevated glutamate levels may be associated with reduced immunological defense against the tumor and that patients with high glutamate levels may therefore have a higher death rate than those with relatively low levels. The latter prediction was confirmed in both groups of tumor patients (Fig. 1). The mean survival of all tumor patients with glutamate levels of $>120$ µ*M* was significantly shorter than that of all patients with glutamate levels of $<120$ µ$^2$M ($P<0.003$, Wilcoxon rank-sum test).

## Discussion

Taken together, our studies revealed that patients with high glutamate levels have, on the average, a lower lymphocyte reactivity and a poorer prognosis than those with low glutamate levels. This correlation per se does not prove a causal relationship between glutamate level and mortality. Unfortunately, we are not aware of any clinically applicable procedure that could be used to reduce the plasma glutamate levels or to test the hypothesis that this strategy may also reduce the death rate. Also, the glutamate level does not appear to be a reliable diagnostic parameter at the individual level, since some patients with low glutamate levels were nonetheless found to survive only briefly. However, the results of these studies provide a striking confirmation of a clinically important prediction from in vitro laboratory data, which have previously shown that elevated extracellular glutamate levels impair functions of macrophages and lymphocytes (Eck and Dröge 1989; Eck et al. 1989). It is conceivable that this inhibitory effect of glutamate on cells of the immune system may have consequences with respect to the host-tumor relationship and to disease progression. Several different manifestations of impaired immune functions in patients with bronchial carcinoma have previously been reported (for review see Manke and Aulenbacher 1985).

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