Amino acids and immune response

*Early observations in underprivileged communities:*

• Inadequate energy intake
• Inadequate protein (N) intake
• Specific nutrient deficiencies (e.g., amino acids)

weakened host defense mechanisms
increased risk for infections

Chandra, 1991
Amino acids and immune response

*Traditional approach:*

- Physiological role of the individual amino acid in immune response can be explained by their nutritive value
  - indispensable (essential) amino acids
  - dispensable (non-essential) amino acids (as N source)
- Covering specific nutritive requirements ensures adequate proliferation of immune cells and their cytotoxic functions

*Amino acids and immune response*

*Current approach:*

- Apart from their nutritive value, high intake of individual amino acids can *modulate* cellular immune response in stressed patients
- Potential candidates:
  - arginine
  - glutamine
  - cysteine/taurine
  - glycine (??)    and others.....
Immune nutrition – amino acids
structure of presentation

• Immune modulating properties
  ➢ mechanism(s)
  ➢ experimental and clinical studies
• Recommendations for clinical routine

Arginine and immune cell function

Endogenous arginine is needed for

• proliferation of lymphocytes  Efron et al, 1991
• induction of T-cell functions  Efron & Barbul, 1998
• synthesis of nitric oxide (NO)  Nathan & Xie, 1994; Albina, 1996
• increase of PMN phagocytosis  Efron & Barbul, 1998
• increase of NK and LAK cytotoxicity  Efron & Barbul, 1998
  and others.....
Postulated mechanism of arginine action

In-vivo effects of high dose arginine supplements – experimental studies

- Enhanced peripheral blood lymphocyte mitogenesis
- Suppression of tumor growth
- Alteration of tumor infiltrating lymphocyte phenotype
- Enhanced wound collagen deposition and breaking strength
- Potent endocrine secretagogue
- Stimulation of tumor growth

Adopted from Efron & Barbul, 1998
Arginine-supplemented diets improve survival in gut-derived sepsis and peritonitis (mice model)

Transfusion + Gavage + Burn

![Graph showing survival rates]

Gianotti et al., 1993

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In-vivo effects of supplemental arginine – early clinical trials

*oral supplementation with 20-30 g Arg/d:*

- increases blastogenesis in peripheral lymphocytes in healthy subjects  
  Barbul, 1990
- prevents the postoperative decrease of lymphocyte blastogenesis  
  Daly et al., 1988
- increases the mitogenic response of T-cells in HIV+ patients  
  Barbul, 1990
Supplemented enteral formulas and immune response

After injury or surgery, enteral formulas enriched with arginine, RNA, and ω-3 fatty acids and with different micronutrient pattern were associated with

- improved host defense mechanisms
  (eg. phagocytosis ability, respiratory burst, inflammatory response)
- improved patient outcome


Open questions:

- Which is/are the effective substrate(s)?
- Which amounts are needed?

Supplemented enteral formulas - arginine

CAVE!

- “Given the potential harm (increased mortality) associated with the use of diets supplemented with arginine and other nutrients in septic patients…, the committee decided to recommend against their use in critically ill patients."

Heyland et al: Canadian guidelines, JPEN 2003
Glutamine and immune cell function

Endogenous glutamine is needed for

- promotion of lymphocyte proliferation
  - glutamine is used as energy fuel and nitrogen donor

- attenuation of the elaboration of pro-inflammatory mediators
  - effects are modulated by glutathione

- upregulation of anti-inflammatory factors
  - glutathione – structure and function

Glutathione – structure and function

- glutamyl-cysteinyl-glycine
  - (Glu-Cys-Gly, GSH)

  synthesis from glutamate, cysteine and glycine

Selected functions:

- Elimination of peroxides (glutathione peroxidase):
  \[ 2 \text{GSH} + R_2\text{O}_2 \rightarrow \text{GSSG} + 2 \text{ROH} \]

- Synthesis of leukotrienes (glutathione-S-transferase):
  \[ 2 \text{GSH} \rightarrow \text{Cys-leukotrienes} \]
Glutamine and immune response – clinical studies in stressed patients

- Total lymphocyte, T lymphocyte, total CD\textsubscript{4} and CD\textsubscript{8} counts
  Ziegler et al. 1992, 1994, Morlion et al. 1998
- Increased T lymphocyte proliferative responses
  O’Riordain 1994, 1996
- Improved T-cell DNA synthesis
- Improved intestinal barrier function
  Neu et al. 2002
- Greater leukocytosis after BMT and major operations
  Jacobi et al. 1997
- Pro-inflammatory cytokines↓, anti-inflammatory cytokines↑
Glutamine (dipeptide) increases secretion of Cys-leukotrienes from PMN in postoperative patients

TPN (n=5) supplemented with Ala-Gln (blue) or conventional (yellow); mean ± SEM; sign. between groups: ***p<0.001, sign. with preop: *p<0.05

Cysteine and immune cell function

Endogenous cysteine is needed for

• Stimulation of lymphocyte proliferation
  Dröge et al, 1991

• Activation of cytotoxic T-cells
  Grimble, 1993

• Modulation of hepatic glutathione synthesis

• Inhibition of NFkB expression in T-cells
Parenteral cystine peptides increase plasma glutathione in experimental rats

GSH [nmol/ml]

- Group I: controls
- Group II: met deficient
- Group III: supplemented with bis-glycyl-cystine
- Group IV: supplemented with bis-alanyl-cystine (mean +/- SD 7th day)

Glycine and immune cell function

First in vitro-observations:

- Reduction of pro-inflammatory TNF-β and IL-1β and increase of anti-inflammatory IL-10 production after LPS exposure in human monocytes
  - Blockade of Ca²⁺ influx? Spittler et al. 1999, Roth et al. 2003
- Inhibition of CD3-stimulated proliferation of lymphocytes modulated by IL-2 Stachlewitz et al. 2000
Glycine and immune response – clinical studies

Indirect evidence from studies with glycyl-glutamine:

- Perioperative supply of glycyl-glutamine but not alanyl-glutamine diminished the surgery-induced period of immunosuppression (rapid restoration of TNF-α production)  
  Exner et al. 2003

- Postoperative glycyl-glutamine infusion partially prevents surgery-induced HLA-DR expression on monocytes  
  Spittler et al. 2001

Is glycine the true effector?

Recommendations for clinical routine

Glutamine:

- in stressed situations classified as indispensable amino acid and should be part of nutritional efforts (glutamine-enriched regimen; tentative requirements: 0.15 – 0.45 g/kg)

  - no or insufficient supply will impair immune cell function (nutritive approach)

  - supplements (physiological amounts) motivated when nutrition therapy does not cover needs
Recommendations for clinical routine

Arginine and glycine:
• no signs of depletion and/or higher needs in stressed situations
• nutritive amounts present in all commercial preparations for clinical nutrition
• dose-response data are not available; nutritive amounts sufficient to ensure metabolic functions
• use of additional supplements is not motivated

Recommendations for clinical routine

Cysteine:
• only in specific pathological situations conditionally indispensable (e.g. liver failure; tentative requirement: 20 mg/kg)
• precursor methionine present in all commercial preparations for clinical nutrition
• way of administration (e.g. parenteral) influences transsulfuration of methionine to cysteine
• necessity to give cysteine itself?
• final conclusions concerning supplements can not be drawn due to lack of data
Conclusion and future aspects

- Individual amino acids like arginine, glutamine, and cysteine possess “immunomodulating” functions.
- Adequate intake of these amino acids in the frame of clinical nutrition may beneficially influence cellular immune response in various patient groups (nutritive approach).
- Rationale for high-dose supplementation of single amino acids exceeding nutritive amounts is not available.

Immunonutrition in ICU – systematic reviews and meta-analyses

Flooding of information:
- Beale RJ et al, Crit Care Med 1999
- Heyland et al, JAMA 2001
- Montejo et al, Clin Nutr 2003
- Heyland et al, JPEN 2003
Common statements:

- Heterogeneity of results (variations with respect to enteral formula, patient population, intervention, methodological quality) hinders formulation of final recommendations for ICU patients.
- Grade B and C recommendation for use of diets enriched with pharmaconutrients only for defined subgroups.
- More studies with only one pharmaconutrient are necessary.