Interactions between nutrition and immunity in anorexia nervosa: a 1-y follow-up study\textsuperscript{1-4}

Ascensión Marcos, Pilar Varela, Olga Toro, Irene López-Vidriero, Esther Nova, Diana Madruga, José Casas, and Gonzalo Morandé

ABSTRACT  
Nutritional status and immunocompetence were evaluated in 15 patients suffering from anorexia nervosa in comparison with a control group (n = 15). After 1 y, data from six phases of the study were evaluated: immediately after admittance to the hospital (AN1), after 1 mo (AN2), after 2 mo (AN3), after 3 mo (AN4), after 6 mo (AN5), and after 1 y (AN6). Patients recovered weight from AN4 until AN6 although, according to body mass index values, all patients had low weights during the 1-y follow-up. Likewise, leukocyte and lymphocyte values were borderline and lower in patients in all phases tested than in control subjects. All lymphocyte subpopulations were lower in AN1 and AN2 patients (inpatients) than in control subjects, except for CD19 cells, which remained unmodified. There seemed to be a recovery of lymphocyte subsets after hospitalization in AN3 and AN4 patients (outpatients), except for CD57, which remained below control values. However, there was a global decrease of the lymphocyte subsets in AN5 and AN6. Ratios of CD4 to CD8 cells were not altered but the ratio of CD2 to CD19 cells was lower in all phases except AN6. Moreover, cell-mediated immune function was impaired and none of the patients showed normal responses. Thus, despite the slight weight increase found in AN4, AN5, and AN6 and the apparent cell subset recovery after hospitalization, these results suggest a greatly depleted nutritional status that remained during the whole year in all patients. Am J Clin Nutr 1997;66:485S–90S.

KEY WORDS  
Anorexia nervosa, follow-up, nutritional status, lymphocyte subsets, immune function, delayed-hypersensitivity skin test, females

INTRODUCTION  
Anorexia nervosa is a psychiatric syndrome characterized by a marked reduction of food intake in the obsessive pursuit of thinness (1). Eating disorders are considered to be multicausal and multidimensional disorders (2). In fact, the underweight phase involves nutritional deficiencies, which are associated with abnormalities in neuroendocrine function (3–5), stress, and depression (6). The presence of multiple nutritional deficiencies is a well-recognized cause of immunodeficiency in humans, leading most consistently to depressed cell-mediated immunity (7).

Many studies have shown that the neuroendocrine system can control immune functions (8). Thus, the diverse endocrine changes reported in anorexia, affecting not only sex hormones but also cortisol, opioid activity, and other neurotransmitters (9), may lead to inevitable changes in the immune system. Stress and depression may also affect immune responses by as yet undefined mechanisms (10).

Although all the processes that occur in anorexia nervosa have a profound effect on immune functions (11–14), the immunologic aspects of the disease have received little attention. Few studies have focused on immune responses in anorexia nervosa and results are controversial (7, 15, 16). Although few studies have shown functional cellular abnormalities in patients with anorexia nervosa (7), others have found normal or above-normal T lymphocyte populations and unimpaired proliferative responsiveness to mitogenic stimulation (16).

The complexity of the interaction between nutrition and infection is well recognized. Patients with protein-energy malnutrition are typically more susceptible to infections (17). Studies of malnourished subjects have disclosed disturbances of cell-mediated and humoral immunity (18, 19). However, increased vulnerability to infections is less common in anorexia nervosa.

Nevertheless, the association between refeeding and infection has been suggested in the past. On the basis of certain clinical observations, it has been suggested that starvation may suppress and refeeding activate certain infections (20). Because there are few studies carried out on the evolution of the illness, the aim of this work was to evaluate in a 1-y follow-up the nutritional status by immunologic assessment of patients suffering from anorexia nervosa in comparison with a healthy control group.

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SUBJECTS AND METHODS

Fifteen female inpatients (ranging in age from 10 to 20 y) diagnosed at the Hospital General de Móstoles, Madrid, who met DSM-III-R criteria for anorexia nervosa (21) were included in this study. A 1-y follow-up was carried out on these patients. Six different phases of the illness were taken into account: immediately after admittance to the hospital (AN1), after 1 mo (AN2), after 2 mo (AN3), after 3 mo (AN4), after 6 mo (AN5), and after 1 y (AN6).

The control group consisted of 15 healthy young female students from a school, who were matched with the patients by age and sociocultural level. The control group was free of either psychiatric or somatic disease. All subjects (control subjects, patients, and the medical staff) gave informed consent once the purpose and nature of the study was explained. The procedures followed were in accord with the Helsinki Declaration as updated in Tokyo, 1975, and revised in 1983.

The assessment of patients and control subjects included the following anthropometric measurements: age, weight, height, and body mass index (BMI, in kg/m²) (22). Blood samples were taken after 12–15 h of fasting and after 30 min rest. Leukocyte and lymphocyte counts were assessed by routine analytic methods (Coulter Counter, Hialeah, FL).

Lymphocyte subpopulations were assessed as follows. Whole-blood samples (100 µL) were incubated with 10 µL of appropriate, titered monoclonal antibodies (Coulter Clone, Coulter Corporation) at 4 °C for 10 min to evaluate the following lymphocyte subsets: CD2 (pan T cells), CD3 (T cells), CD4 (helper T cells), CD8 (cytotoxic or suppressor T cells), CD19 (B lymphocytes), and CD57 (natural killer cells) by flow cytometry. Each sample was processed with the Immunoprep EPICS lymphocyte preparation system. The Immunoprep reagents include a lysis agent for elimination of erythrocytes, a stabilizer for the leukocytes, and a fixative to maintain sample integrity (23). The control samples were incubated with purified phycoerythrin-labeled mouse and fluorescein isothiocyanate–labeled mouse immunoglobulin G1. The fluorescence of the subsets was analyzed with a Facstar Plus dual-laser cytometer (Becton Dickinson, Sunnyvale, CA). Forward light-scatter intensity combined with ring-angle scatter was analyzed by using the appropriate software.

Delayed dermal hypersensitivity to seven recall antigens was assessed with the Multitest CMI skin test antigen applicator (Merieux Institute Inc. Miami). The seven antigens administered simultaneously by this applicator were tetanus, diphtheria, streptococcus, tuberculin, Candida, Proteus, and Trichophyton, as well as a control (glycerin) injection. Reactions were measured 48 ± 2 h after injection by measuring induration diameter (in millimeters). Induration of ≥ 2 mm was considered a positive reaction. “Score” is defined as the sum of indurations for positive responses (24). Anergy (score = 0), relative anergy (score = 1 positive response), hypoergy (score = < 5 mm), low response (score = 5–10 mm), and normal response (score = > 10 mm) were defined according to the criteria of Jaurrieta et al (25) for Spanish females.

All results are expressed as means ± SDs. Results were analyzed by one-way analysis of variance (repeated-measures ANOVA) to look at the changes over time for the anorexia nervosa group. Previously, the F statistic was used to test the assumption of homogeneity of variances. When significant differences were found with ANOVA, individual comparisons within anorexia nervosa subjects at admission and at different elapsed times were performed with Student’s t test (paired data). Comparisons between the control and each of the six periods were performed with Student’s t test (unpaired data). Under the assumption of equal variances, the t statistic (two-tailed) was computed using the pooled variance estimate. Under the assumption of unequal variances, the t statistic was computed using the separated variance estimate. Statistical analysis was performed by using the SAS computer program (P < 0.05) (SAS Institute Inc. Cary, NC).

RESULTS

The anthropometric measurements tested are summarized in Table 1. When all groups of anorexia nervosa patients were compared with control subjects, no significant differences were observed in age or height. Weight values were lower in all anorexia phases tested than in the control group, although there was a significant increase 3 mo after admission (AN4) compared with immediately after admission (AN1). This increase was maintained in the following phases (AN5 and AN6). Similar results were observed for BMI; in all anorexia phases, BMI was lower than in control subjects. BMI increased significantly from AN1 to AN2 and remained unmodified until the last phase of the follow-up (AN6).

White blood cell counts as well as lymphocyte counts and percentages are summarized in Table 2. There were no differences in leukocyte or lymphocyte counts in all phases tested and values for all anorexia phases were lower than in the control group. However, lymphocyte percentages were lower in AN5 and AN6 than in both AN1 and in control subjects.

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
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<tbody>
<tr>
<td>Anthropometric indexes in control subjects and in anorexia nervosa patients during follow-up( ^{1} )</td>
</tr>
<tr>
<td>---</td>
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<tr>
<td>Age (y)</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>Height (cm)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
</tbody>
</table>

\( ^{1} x ± SD; n = 15 \) in each group. Anorexia nervosa (AN) groups were as follows: AN1, immediately after admittance; AN2, after 1 mo; AN3, after 2 mo; AN4, after 3 mo; AN5, after 6 mo; and AN6, after 1 y.

\( ^{2} \) Significant differences among AN groups when comparing values at the different elapsed times (repeated-measures ANOVA, time effect \( P < 0.05 \)).

\( ^{3} \) Significantly different from control, \( P = 0.05 \) [Student’s t test (unpaired data)].

\( ^{4} \) Significantly different from AN1, \( P = 0.05 \) [Student’s t test (paired data)].
Peripheral blood lymphocyte subsets were significantly different between anorexia nervosa patients and control subjects. The CD2 subset was lower in all phases tested than in the control group, except in AN3, 2 mo after admission, for which patients had values similar to those of control subjects. Values for both CD3 and CD4 cells were lower in AN1, AN2, AN5, and AN6 than in the control group, although there were no differences among all the values of the phases. Values for CD8 cells in AN1, AN2, and AN5 were significantly lower than in control subjects. CD57 cells were much lower during all of the follow-up study than in the control group. The CD19 subset seemed to behave differently from the rest of the lymphocyte subpopulations because there were no significant differences between the phases tested and the control group, except for AN6, in which the CD19 subset was significantly lower than in control subjects. In addition, a decrease was observed in AN2 and AN6 in comparison with AN1.

In relation to the ratio of CD4 to CD8 cells (CD4:CD8), there were no differences when all phases were compared each other and with the control group, although there was a tendency for the ratio to decrease in AN6. However, the ratio of CD2 to CD19 cells (CD2:CD19) was lower in AN1, AN2, AN4, and AN5 than in the control group, but no changes were found in AN6. Thus, values for AN6 were significantly different from those for AN1, AN4, and AN5.

For lymphocyte subset percentages (Table 3), the profile was different from that observed for lymphocyte counting. Lower percentages of CD2 and CD57 cells were found in AN1 and during the whole follow-up, respectively, together with higher percentages of CD3 cells in AN3 and AN4, CD4 cells in AN3, CD8 cells in AN6, and CD19 cells in AN1, AN3, and AN5. The percentages of most of the lymphocyte subsets remained unmodified in all phases tested. Exceptions were for CD19 and CD57: the percentage of CD19 cells decreased between AN1 and AN6 (14.95 ± 5.59% compared with 10.07 ± 3.87%) whereas the percentage of CD57 cells increased (4.26 ± 3.15% compared with 6.62 ± 3.52%).

When cell-mediated immune function was evaluated through use of a delayed-hypersensitivity skin test, a reduced response was found in all anorectic patients during the whole follow-up (Table 4). Thus, there were few changes during the phases tested in the current study. Only the control group showed a normal response of 100%. Moreover, normal responses were not seen in those phases in which lymphocyte subsets seemed to be slightly recovered (AN3 and AN4); on the contrary, 80% of anorexia nervosa patients had relative anergy in AN3 and 25% of them showed anergy in AN4.

### DISCUSSION

Anthropometric indexes mostly showed a sharp nutritional depletion in the young females suffering from anorexia ner-
TABLE 4
Response to delayed-hypersensitivity skin test in control subjects and in anorexia nervosa patients during follow-up

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>AN1</th>
<th>AN2</th>
<th>AN3</th>
<th>AN4</th>
<th>AN5</th>
<th>AN6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anergy (score = 0) (%)</td>
<td>0</td>
<td>23</td>
<td>13</td>
<td>0</td>
<td>25</td>
<td>46</td>
<td>17</td>
</tr>
<tr>
<td>Relative anergy (score = 1 positive response) (%)</td>
<td>0</td>
<td>6</td>
<td>13</td>
<td>80</td>
<td>25</td>
<td>23</td>
<td>11</td>
</tr>
<tr>
<td>Hypoergy (score = &lt; 5 mm) (%)</td>
<td>0</td>
<td>6</td>
<td>6</td>
<td>20</td>
<td>8</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Low response (score = 5-10 mm) (%)</td>
<td>0</td>
<td>47</td>
<td>44</td>
<td>0</td>
<td>42</td>
<td>16</td>
<td>33</td>
</tr>
<tr>
<td>Normal response (score = &gt; 10 mm) (%)</td>
<td>100</td>
<td>18</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>No positive response to 7 antigens</td>
<td>4.78 ± 0.49*</td>
<td>2.35 ± 1.69*</td>
<td>2.50 ± 1.71*</td>
<td>1.00 ± 0.00*</td>
<td>1.58 ± 1.24*</td>
<td>1.31 ± 1.70*</td>
<td>2.11 ± 1.32*</td>
</tr>
<tr>
<td>Score (mm)*</td>
<td>14.42 ± 1.19</td>
<td>6.75 ± 5.26</td>
<td>6.70 ± 5.12</td>
<td>2.10 ± 0.22</td>
<td>3.77 ± 3.21</td>
<td>4.44 ± 6.64</td>
<td>5.76 ± 3.98</td>
</tr>
</tbody>
</table>

1 Anorexia nervosa (AN) groups are defined in Table 1. See methods section for definition of scores.
2 Significant differences among AN groups when comparing values at the different elapsed times (repeated-measures ANOVA, time effect P < 0.05).
3 * ± SD.
4 Significantly different from control, P ≤ 0.05 [Student’s t test (unpaired data)].
5 Significantly different from AN1, P ≤ 0.05 [Student’s t test (paired data)].

vosa: both weight and BMI values were low for the patients’ ages and heights (22). BMI values in the anorexia nervosa patients during the 1-y follow-up in the current study are within the low weight range according to the Llewellyn-Jones and Abraham (26) criteria. Although some patients showed emaciation, the percentage with a BMI < 15 decreased from 37.5% to 14.3% in AN1 and AN6, which could point to a slight recovery 1 y after admission. The slight, although significant, BMI increase from AN2 remained unmodified during the 1-y follow-up, leading to a plateau that seems to be difficult to avoid.

Although lower values for leukocytes and lymphocytes were found for all patients in comparison with control subjects, values were within the normal range but in the borderline established by Vives (27). Thus, in the current study, 22% and 10% of the females suffering from anorexia nervosa, both at admission and 1 y later, respectively, had <4 x 10⁹ leukocytes/L blood. Similar results were observed previously by other authors (28–30). No differences were found in lymphocyte percentages between the anorexia nervosa groups and the control subjects, although the AN5 group (6 mo after admission) had lower values than did the control group. On the other hand, in a previous work, a relative lymphocytosis was found in anorexia nervosa patients (31).

The behavior of all T lymphocyte subpopulations tested was similar. At the beginning of the study, while the patients were in the hospital (AN1 and AN2), T cell values were low, which may involve depleted cell-mediated immunity according to the results found by Chandra (32). During AN3 and AN4, T cell values might be recovered, which seems to be linked to the period when the patient leaves the hospital. An exception to this is CD2 cells, which decrease again in AN4 in comparison with values in control subjects. These results could suggest a recovery of cell-mediated immunity after the hospital stay. However, in AN5 and AN6, when the patients stay at home for a long period of time (between 6 mo and 1 y after admission to the hospital), a relapse in cell-mediated immunity seems to occur.

Studies of malnourished subjects have disclosed disturbances of cell-mediated and humoral immunity (18, 19). The literature concerning cell-mediated immunity in anorexia nervosa is controversial (7, 15, 16, 33). Impaired cell-mediated immunity in terms of a delayed-hypersensitivity reaction, lymphocyte transformation response to T mitogens, and decreased numbers of T3 and T4 lymphocyte subpopulations has been described in anorexia nervosa (7). In addition, cell-mediated cytotoxicity was found to be markedly low in anorexia nervosa patients compared with control subjects (34). However, Golla et al (16) and Armstrong-Esther et al (15) found no significant differences between anorexia nervosa patients and control subjects with respect to lymphocyte subpopulations. It was therefore suggested by Golla et al (16) that the maintenance of a relatively intact cell-mediated immune system in anorexia nervosa may be an important factor distinguishing these patients from patients with other forms of protein-energy malnutrition.

Nevertheless, CD19 cells remained unmodified in all phases evaluated except AN6, during which CD19 cells decreased significantly compared with values for control subjects and during AN1. The data related to B cells in eating disorders are controversial; in earlier studies an increase in B cells was found in bulimia nervosa patients compared with control subjects (35). In addition, in the present study, natural killer cells were lower in anorexia nervosa patients than in control subjects during the whole follow-up. This could be used as an index of malnutrition (36) even at a subclinical level, especially in AN3 and AN4, when there seemed to be an immunologic recovery.

According to Schattner et al (33), cell-mediated cytotoxicity was markedly lower in anorexia nervosa patients than in control subjects. The authors made similar observations in acute starvation and others have also noted depressed natural killer cell activity in protein-energy malnutrition, which was corrected by proper dietary intake (37). This apparently does not lead to a greater susceptibility in anorexia nervosa patients to infections or neoplasia (7) and does not appear to be due to a defective interferon system because this has been shown to be normal (33). These results are somewhat contradictory with reports of enhancement of natural killer cytotoxicity by tumor necrosis factor as well as by β-endorphins (38, 39), which were also elevated in some of the anorexia nervosa patients (37).

CD4:CD8 and CD2:CD19 are good indexes of nutritional status (34, 36). In the current study, CD4:CD8 remained unmodified in all phases evaluated in anorexia nervosa patients, which could mean an enhanced nutritional status compared with that observed in a previous study (31). Comparing both studies, the illness evolution period before patients were admitted to the hospital was longer in the first study (1.5 y) than
in the current study (between 3 and 6 mo). Thus, it could be suggested that early therapy intervention plays an important role in the evolution and prognosis of the illness. However, because CD2:CD19 values were lower in patients than in control subjects, some symptoms of malnutrition (36) seemed to still be present in these patients. Likewise, cell-mediated immune function evaluated through use of the delayed-hyper-sensitivity skin test (24) appeared to be impaired in all anorexia nervosa groups tested compared with control subjects because responses to the test were depressed (25).

Whereas few studies have shown functional cellular abnormalities in patients with anorexia nervosa (7), others have shown normal or above-normal T lymphocyte populations and unimpaired proliferative lymphocyte responsiveness to mitogenic stimulation (16). This outcome agrees with the conclusions of some authors who pointed out that nutritional deficiencies, even at a subclinical level, may modify the immune system, mainly cell-mediated immunity (7, 40).

According to Bessler et al (41), a stimulator may be present in the serum of anorexia nervosa patients. Such a stimulator may compensate for the lower capacity of patients’ peripheral blood mononuclear cells to produce interleukin 2. Such a mechanism provides a relatively normal immune response and may explain why anorexia nervosa patients are not prone to infections despite lower cytokine production by their peripheral blood mononuclear cells. In conclusion, even though the patients recovered body weight from AN4 through AN6, it was not sufficient to maintain a desirable nutritional status because they did not reach an adequate BMI and their immunologic indexes were lower than in control subjects during the whole 1-y follow-up. Although the outcome of anorexia nervosa remains uncertain, early treatment seems to be an important step in the follow-up of the illness.

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REFERENCES