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Decreases in Mean Hemoglobin and Serum Albumin Values in Erythema Nodosum Leprosum and Lepromatous Leprosy[1]

Thomas H. Rea[2]

Anemia ([18]) and hypoalbuminemia ([16]) are concomitants of many chronic diseases, whether infectious, inflammatory or neoplastic in origin. These changes are the consequence of the illnesses, but they may have prognostic importance ([36]) or be of sufficient severity to be clinically important. In leprosy two kinds of anemia are recognized often. Of these, a dapsone-induced hemolysis is the most common. For example, in their retrospective study of 100 patients receiving dapsone, Byrd and Gelber ([4]) found a fall in hemoglobin (HGB) of 1 g/dl or more in 83% of patients and an average fall of 1.94 g/dl in the entire group. The other common anemia, usually called the anemia of chronic disease (ACD), but pathophysiologically better characterized as "cytokine mediated anemia," is defined by a low serum iron in the presence of ample iron stores ([18]), and has been well documented in lepromatous leprosy (LL) and borderline lepromatous (BL) patients ([32]). Characteristically normochromic and normocytic, this anemia is associated with normal to low iron binding protein levels with low saturation, normal to low serum iron concentration, but normal or elevated serum ferritin values, a surrogate for iron stores ([18]). Hastings, *et al.* ([11]), found statistically significant decreases in mean HGB and hematocrit (HCT) values in patients with erythema nodosum leprosum (ENL) who had been treated with corticosteroids. The fall was noted when the patients entered a 4 day long "washout" period following cessation of corticosteroid treatment, while awaiting initiation of thalidomide therapy. Furthermore the fall continued during the 3–4 day period when thalidomide had induced a remission in the ENL. It was concluded that the ENL-associated fall was neither hemolytic nor a failure of erythropoiesis, but had an unknown mechanism. Because ENL is common in LL patients, an ENL-associated anemia may also be common. In leprosy hypoalbuminemia has also been recognized in some LL patients, as well as in some patients with ENL ([10, 14]).

A recently observed dramatic and synchronous fall in both HGB and serum albumin (SA) values in a patient with relapsing ENL prompted a retrospective study on these parameters in our patient population, as reported herein.

MATERIALS AND METHODS

All the subjects in this study were outpatients in the Hansen's Disease Clinic of the Los Angeles County/University of Southern California Medical Center. Patients were classified according to the criteria and nomenclature of Ridley and his colleagues ([28]). Criteria for the diagnosis of ENL were those previously published ([27]).

Three groups of patients with ENL were identified for study, each by different criteria, in order to reduce the risk of ascertainment bias. One group, Group 1, consisted of the patients started on thalidomide for the treatment of ENL during the decade of the 1990s, who had been receiving conventional antimicrobial therapy, and who had never received corticosteroids before beginning thalidomide. A second group, Group 2, consisted of the patients in the active file who presented with untreated, nonreac-

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[2] Thomas H. Rea, M.D., The Division of Dermatology, Keck School of Medicine, University of Southern California, Los Angeles, California.

Reprint requests to T. H. Rea, M.D., LAC/USC Medical Center, 1200 N. State St., Room 8440, Los Angeles, California 90033, U.S.A.; e-mail: rea@hsc.usc.edu

tional lepromatous leprosy (LL), whether polar or sub-polar, and who were not in Group 1. A third group, Group 3, consisted of patients who had been managed with rifampin in combination with either minocycline or clarithromycin, and who were initially not in reaction. These groups have been kept separate for analysis of probability values.

Excluded from each of the three groups were patients who had other diseases, or were receiving medications, excepting dapsone, which might influence HGB or SA values. In addition, to mitigate the confounding effect of dapsone upon HGB values, patients receiving dapsone who developed ENL before 6 months of dapsone therapy had elapsed also were excluded from study. Also, on any particular day a particular datum sought might not have been obtained, for example eliminating the individual for SA, but not HGB, evaluation.

For the purposes of this study, the laboratory values taken to be reaction-associated were those obtained on the day thalidomide or prednisone was started for management of the reaction. Thalidomide or prednisone was only used after the patient had developed systemic symptoms such as chills, fever, insomnia, anorexia, and fatigue, however long had been the presence, or however many the number of asymptomatic or "incipient" cutaneous lesions of ENL. Thus the time of starting ENL was taken to be the time of onset of systemic symptoms.

The data presented were obtained in the medical center's clinical laboratory as part of routine patient care. The laboratory's normal values for SA were 3.5 g/dl to 5.0 g/dl. Normal HGB values ranged from 11.6 g/dl to 14.9 g/dl for women and 12.9 g/dl to 16.6 g/dl for men. The normal ranges for iron studies were as follows: serum iron 50 μg/dl–160 μg/dl, serum ferritin 30 ng/ml–300 ng/ml in men and 15 ng/ml–150 ng/ml in women, total iron binding capacity (TIBC) 230 μg/ml–410 μg/ml, and percent saturation 15–55.

To determine a possible influence of ENL upon HGB or SA values, control values were obtained from the clinic visits prior to the onset of ENL. In all but one patient each control value was the average of the three immediately preceding values. In the exception only one control value was available for each parameter.

To determine a possible influence of antimicrobial treatment upon HGB or SA values in nonreactional LL patients, the values obtained before treatment served as controls, being compared to those obtained at 6 months or thereabouts (range 5–8 months).

Statistical analysis utilized the Microsoft Excel program, assuming a normal distribution and unequal variances. A "p-value" of less than 0.05, using a one-tail test, was considered to be significant. Whenever possible the "paired two sample for means" method was used.

RESULTS

Mean hemoglobin changes. Statistically significant falls in mean hemoglobin values were found in association with the onset of ENL in all three groups studied, as illustrated in Figure 1A.

In the 38 patients from Group 1 who developed ENL after 6 or more months of antimicrobial treatment (median time 15 months), mean HGB values fell from 13.19 gm/dl to 12.27 gm/dl, or 7.0%, $p = 6.0 \times 10^{-6}$. In 8 patients from Group 2 who had the onset of ENL after 6 or more months of antimicrobial therapy (median time 13 months), mean HGB values fell from 13.40 gm/dl to 11.96 gm/dl, or 10.7%, $p = 0.0015$. In the 8 patients from Group 3 who had received no dapsone when ENL developed, mean HGB values fell from 13.25 gm/dl to 12.48 gm/dl, or 5.8%, $p = 0.0035$.

Among the 17 LL patients who received no dapsone and who also were not reactional, the mean HGB values rose significantly from 12.39 gm/dl, prior to antimicrobial treatment, to 13.39 gm/dl, or 7.8%, $p = 3.1 \times 10^{-6}$ in association with 6 months of combination antimicrobial therapy.

Mean serum albumin changes. Statistically significant falls in mean SA values were found in association with the onset of ENL in both groups studied, as illustrated in Figure 1B. In the 34 patients from Group 1 who developed ENL after 6 or more months of antimicrobial treatment, the mean SA values fell from 4.1 gm/dl to 3.77 gm/dl, or 8.9%, $p = 1.2 \times 10^{-5}$. A decrease in mean SA values of similar magnitude, but of only weak statistical significance, was found in 10 patients from Group 2 who also had the on-

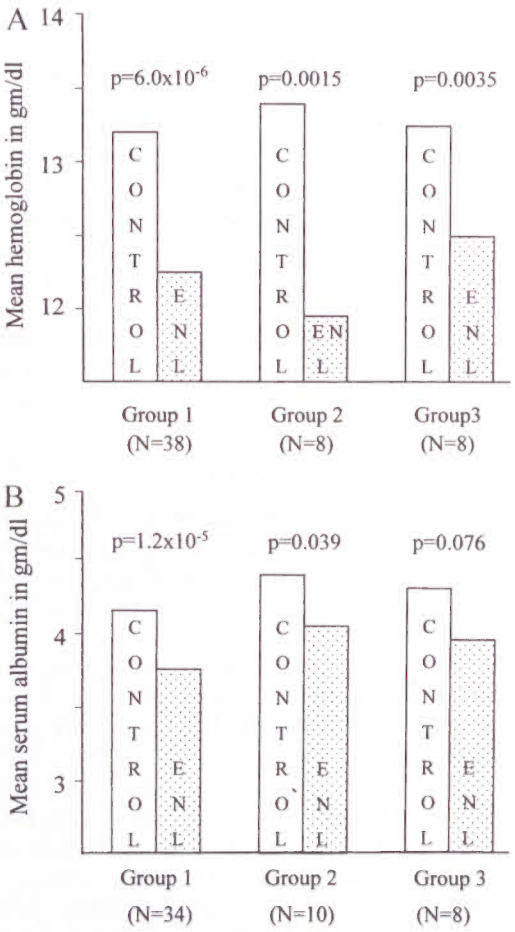

FIG. 1. A. A representation of mean hemoglobin (HGB) values. Control values (open bars) are paired with the values found in association with erythema nodosum leprosum (ENL) (dotted bars) in three groups of patients. B. A representation of mean serum albumin (SA) values. Control values (open bars) are paired with the values found in association with ENL (dotted bars) in two groups of patients.

set of ENL after 6 or more months of antimicrobial treatment, from 4.45 gm/dl to 4.06 gm/dl, or 8.8%, p = 0.039. In the 8 patients from Group 3 who developed ENL, the fall in mean SA values was of similar magnitude, 4.32 gm/dl to 3.97 gm/dl or 8.1%, but was not statistically significant, p = 0.076.

In the 27 patients from Group 1 who developed no reaction in the first 6 months of antimicrobial therapy, the mean SA values rose significantly from 4.21 gm/dl, prior to antimicrobial treatment, to 4.39 gm/dl, or 4.3%, p = 0.0033 in association with this treatment.

Recovery pattern. In an effort to delineate a pattern of recovery for mean SA and HGB values subsequent to the initiation of thalidomide therapy, 11 patients from Group 1 who had the most dramatic fall in both parameters were selected for analysis. The results are summarized in Figure 2, expressed as percent change from control values. Zero-time changes were those on the day thalidomide was started. After one week the mean values had fallen further. In 2–5 weeks recovery was recognizable, but values were still significantly less than controls. Continued recovery was evident at 6–9 weeks, and values no longer differed significantly from controls. At 10–13 weeks mean values for both HGB and SA were virtually identical to controls. All of these patients were receiving dapsone, which was continued after the onset of the ENL. The changes in HGB and SA values appear to be parallel.

Individual changes. Taking together Group 1 and Group 2 patients who presented with nonreactional and untreated LL, i.e., a total of the 40 LL individuals whose data was unencumbered by treatment, 10% or 25%, had HGB values below normal limits, ranging from 9.4 gm/dl to 12.6 gm/dl, and 2% or 5% had SA values less than 3.5 gm/dl.

By the time thalidomide or prednisone was begun for the treatment of ENL in 54 patients shown in Figure 1A, 70% had some fall in HBG values, 44% had a fall of 5% or greater from control values, the largest fall being 34% of the control value, and 52% had values that were anemic (putatively a mixture of dapsone-associated and ENL-associated anemias). The lowest value was 8.6 gm/dl. In this same group, 76% had a fall in SA values, the largest fall being 29% of the control value, and 24% had SA values that were hypoalbuminemic. The lowest value was 1.9 gm/dl.

The temporal dimension. The ENL-associated fall in HGB values was too rapid to be due to inhibition of erythropoiesis, as was demonstrated in the 10 subjects who had data obtained within one week of the onset of ENL and who also had fall of 5% or greater, as summarized in Figure 3A. Similarly rapid falls in SA values, not explainable by inhibition of synthesis, were observed, as summarized in Figure 3B,

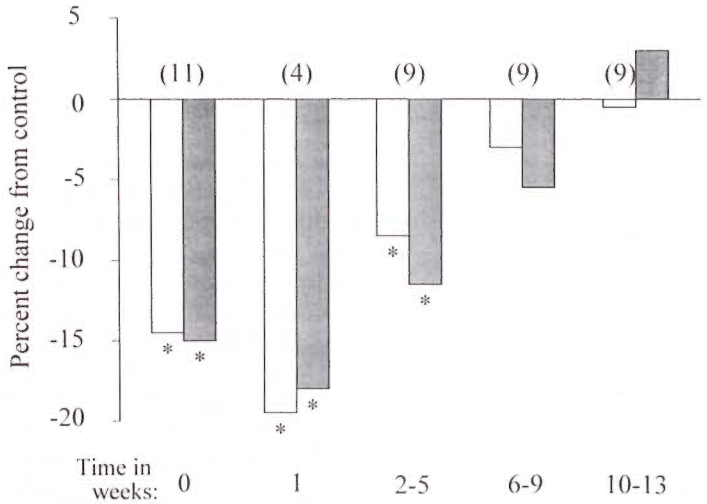

FIG. 2. A representation of the recovery pattern found in 11 patients with large decreases in hemoglobin (dark bars) and serum albumin (open bars) values in association with erythema nodosum leprosum. The changes are plotted as percent of control values. Zero time is the day thalidomide was started. The asterisk (*) indicates a statistically significant change.

which represents 9 of the 10 patients shown in Figure 3A. Comparison of Figure 3A with Figure 3B shows that the magnitude of the fall in HGB values may be greater, equal to, or less than the fall in SA values, but that a fall in the value of one parameter is apt to be associated with a fall in the value of the other.

Clinical importance. In only one patient did the anemia appear to be clinically important, an elderly man with ENL of one week duration who developed signs of congestive heart failure. The low SA values encountered in these patients did not pose clinical problems, but the possibility of contributing to thalidomide-associated pedal edema cannot be excluded. Two patients, each with a brisk fall in HGB concentration, did provoke alarm in two physicians, one incorrectly making a diagnosis of glucose-6-phosphate dehydrogenase deficiency and the other expressing consternation that dapsone was continued despite a dapsone-induced hemolysis.

Iron studies. The serum iron studies were performed on 18 patients in the ENL-focused group, and are summarized in Figure 4. Of the five patients studied before any treatment was started, all had low serum iron values and four had serum ferritin levels high enough to exclude iron deficiency as a cause of their anemia, 200 µgm/dl or greater ([18]). Two of these five presented with untreated ENL. Of the 13 treated patients, 5 had serum iron levels in the low normal range and 5 had ferritin levels in a range that could not exclude the possibility of iron deficiency, 30 µg/dl–200 µg/dl ([18]). These results are consistent with previous studies of iron values in leprosy ([2, 32, 33]).

Erythrocyte indices. ENL-associated changes in HGB, HCT, mean corpuscular volume, and mean corpuscular HGB concentration were examined in 3 small subsets of patients, i.e., those not receiving dapsone, those with large falls in HGB, and those with large falls in SA values. In all 3 groups and in all 4 indices sought, there was a decline in value. However statistically significant changes were limited to HGB and HCT values in all 3 subsets and to mean corpuscular HGB concentration in the group defined by large falls in HGB (data not shown).

DISCUSSION

This study provides substantial, confirmatory evidence that an ENL-associated anemia, or a decline in mean HGB values, is a genuine part of this syndrome. Statistically significant declines in mean HGB values were found in all three groups with ENL. The magnitude of the fall, ranging from 5.8% to 10.7%, is in accord with that found by Hastings, *et al.* ([11]), 6.8% at the

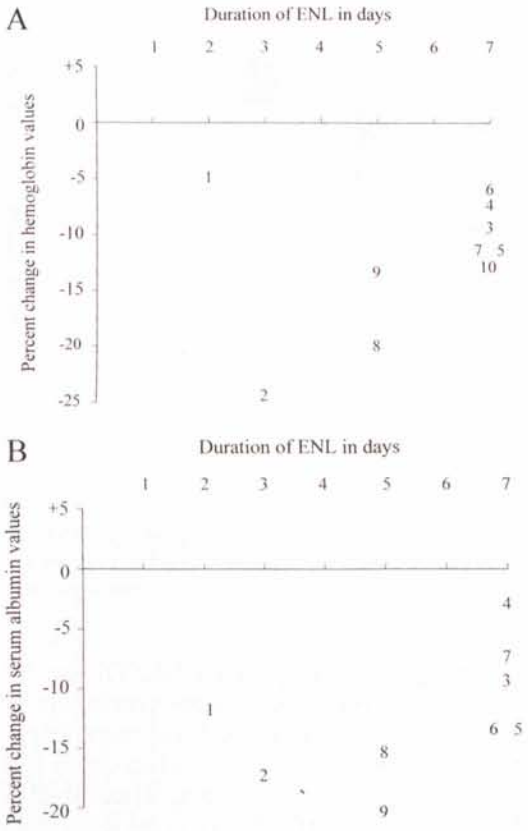

FIG. 3. **A**. The percentage fall in hemoglobin values occurring within 7 days of the onset of erythema nodosum leprosum in 10 patients, each individual indicated numerically. **B**. The percentage fall in serum albumin values occurring within 7 days of onset of ENL in the same patients, each with the same number as in Fig. 3a (no SA value was available in patient #10).

height of the ENL, and 10.6% 3–4 days after starting thalidomide. In both studies the fall in HGB values was rapid, too fast to be explained by inhibition of erythropoiesis. Also both studies found even lower HGB values after starting thalidomide, but, in the present study, the population examined was highly selected and small.

In leprosy, any evaluation of anemia, or a decline in HGB or HCT, is confounded by the common use of dapsone ([4]). The fall in mean HGB values associated with ENL, as reported in this study, is not attributable to concomitant dapsone use, nor is an ENL associated anemia, in and of itself, a reason to discontinue dapsone. Most telling, the fall in mean HGB values was found in the 8 patients not receiving dapsone. Also, in selected patients with the large falls, and in whom recovery was studied, see Figure 3, dapsone was not discontinued but complete recovery occurred. Finally, to mitigate the dapsone influence, patients on dapsone with ENL were excluded from study if their ENL had its onset before receiving 6 months of antibacterial treatment. Thus patients were not studied until two months past the average time taken to reach the nadir of dapsone-induced anemia ([4]) and over half were not studied until after one year of dapsone therapy.

The present study also provides further evidence that ENL is associated with declining SA values, as well as in HGB concentration, and that a large fall in one is likely to be associated with a fall in the other. The failure to find a "significant" fall in SA values in Group 3 is likely a result of the small number of subjects. In addition, this study provides further evidence that nonreactional lepromatous patients after 6 months of antimicrobial chemotherapy have an increase in SA values, and, if not exposed to dapsone, have an increase in HGB values as well.

The present study provides no direct evidence as to the mechanisms underlying the observed changes. Fortunately, the increased information about the biological activities of cytokines has converged with the increased information about cytokines in leprosy, to provide possible explanations for these changes.

Anemias. Among the 18 patients who had serum iron studies, using the criteria of low serum iron, low iron binding protein saturation, and ferritin levels above 200 μg/dl, 9 were considered to definitely have the anemia of chronic disease (ACD). Dapsone induced hemolysis in the thirteen treated patients may confound the interpretation of these studies, but does not alter the conclusion that ACD is common in this patient population as well as in the others previously reported ([32]). The cytokines commonly implicated in the pathogenesis of ACD are interleukin 1 (IL-1), and tumor necrosis factor-alpha (TNF-α), directly inhibiting erythropoiesis or indirectly suppressing erythropoiesis by inhibition of erythropoietin ([18]). Both IL-1 and TNF-α are known to be present in the serum of nonreactional LL patients ([25, 31]), thus providing a reasonable, pathophysiologic ex-

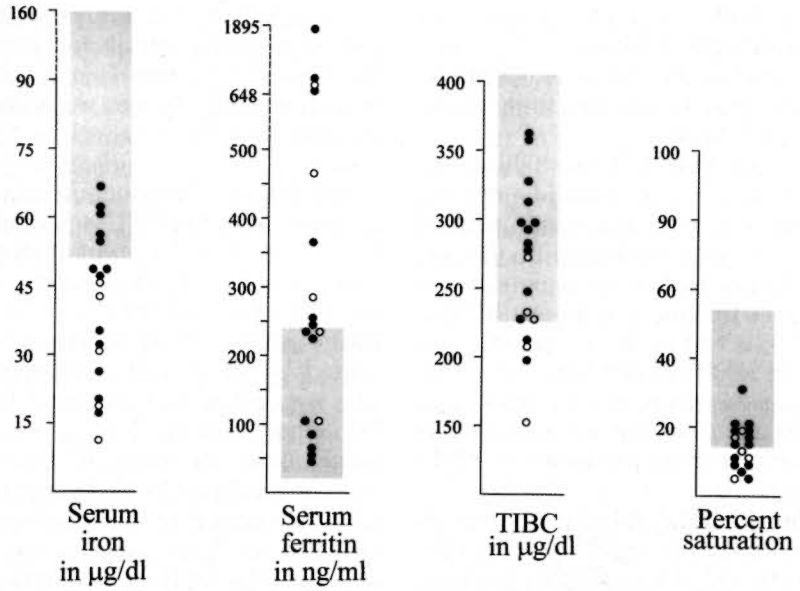

FIG. 4. A representation of serum iron studies. The open circles are values for untreated patients; the closed are for treated patients. The gray rectangles represent the normal range. Total iron binding capacity (TIBC).

planation for this common anemia in such individuals.

Two cytokines, TNF-α and IL-6, offer alternative, or collaborative, explanations for the ENL-associated anemia. TNF-α is known to achieve high serum levels in ENL ([25, 29–31]), thus suggesting TNF-α mediated inhibition of erythropoiesis as a conceivable explanation for the ENL-associated anemia.

IL-6 has been found to be present in the serum of LL patients and was found to rise significantly with ENL, a six-fold increase in mean values ([29, 30]). In addition, two groups have reported increased levels of mRNA coding for IL-6 in ENL tissue specimens, as compared with the lower amounts in LL tissues ([19, 37]). Also found was mRNA coding for IL-6 in peripheral blood mononuclear cells (PBMCs) from patients with ENL and reversal reactions ([19]). Furthermore, in two studies, the *in vitro* production of IL-6 from stimulated PBMCs was greater in cells from lepromatous than tuberculoid subjects ([15, 22]).

IL-6 is also associated with anemia. For example, in humans, *in vivo* infusions of IL-6, whether intravenous ([20, 34]) or subcutaneous ([23]), have resulted in anemia. Blocking studies provide yet another type of evidence. In rheumatoid arthritis patients with ACD, the use of a chimeric monoclonal antibody blocking TNF-α was associated with increased HGB, but decreased IL-6 levels ([5]). In a case report of a patient with Castleman's disease, a disorder with manifestations attributable to overproduction of IL-6, treatment with a humanized anti-IL-6 receptor antibody was associated with improvement of the anemia ([21]). Of particular interest are two studies attempting to define the mechanism of IL-6 mediated anemia, both concluding that the anemia occurs as a result of expanded plasma volume without change in red cell mass ([1, 20]), that is to say, a dilutional anemia. This change was rapid, being detectable at 3 days. Thus a competing explanation is offered for understanding ENL-associated anemia. A dilutional anemia is particularly attractive because the ENL-associated anemia may be of rapid onset, as indicated in the study by Hastings, *et al.* ([11]), as well as our findings summarized in Figure 3A, sooner than would be predicted by inhibition of erythropoiesis. Also Hastings, *et al.* ([11]), found no decrease in reticulocytes. Furthermore, a role for IL-6 in the pathogenesis of ENL is consistent with other ENL phenomena such as the high levels of serum C-reactive protein ([8, 12]) and serum amyloid-associated protein ([12, 17]), IL-6 being a potent hepatic stimulant for the

production of both ([13]). *In vitro* IL-6 stimulates keratinocyte proliferation ([9, 24]) and thus may be involved in the increased thickness of the nucleated epidermis found in the skin lesions of ENL ([26]).

Hypothesizing an IL-6 induced dilutional anemia in ENL does not exclude a role for TNF-α. In humans, systemic administration of TNF-α ([13, 35]), or its leakage into the systemic circulation following isolated limb perfusion in the treatment of inoperable malignancies ([6]), is followed by a prompt rise (beginning in less than one hour) in serum IL-6. Thus, as members of a cytokine cascade, TNF-α and IL-6 may act sequentially and collaboratively to produce the ENL-associated anemia.

Hypoalbuminemia. A fall in SA can result from a number of factors, including decreased synthesis, increased catabolism, hemorrhagic or exudative loss, kidney or gut loss, increased plasma volume, decreased lymphatic return, and increased capillary permeability ([16]). Of these, increased capillary permeability is the major cause of lowered SA values in disease and injury ([7, 16]), and appears relevant in ENL, where proinflammatory cytokines such as TNF-α and IL-6 are present. Decreased synthesis is another possibility, because in the acute phase response, an IL-6 mediated series of events ([13]), albumin synthesis is decreased; however the response of albumin is biphasic, decreased synthesis being followed by an increase, thus making the role of IL-6 difficult to interpret ([16]). Similarly, renal loss, if severe, could be an explanation for low serum values, but, unless part of an overt nephritis, is usually mild, if present at all (unpublished observations). Increased plasma volume, putatively IL-6 mediated, is also a plausible explanation.

The evidence linking IL-6 to low SA values is similar to that linking IL-6 to anemia. For example, in the two studies reporting that the mechanism of anemia resulting from intravenous infusion of IL-6 was increased plasma volume, also reported decreased SA values in the same subject ([1, 20]), occurring too soon to be a result of decreased synthesis. Furthermore, one of the reports of IL-6 elevations secondary to leakage of TNF-α from isolated limb perfusions, also found reduced SA values ([6]). In addition, in one patient with Castleman's disease, where IL-6 overproduction is crucial to its pathogenesis and where hypoalbuminemia has been found, the use of an anti-IL-6 antibody was associated with increased SA values as well as higher HGB levels ([21]).

Inferences. The anemia and hypoalbuminemia found in ENL is perhaps mediated by TNF-α stimulation of IL-6 production. That a common mechanism, or parallel mechanisms, may mediate the decline in mean values of both HGB and SA is supported by the parallel changes observed. The hypoalbuminemia found in ENL may be understood as having several likely mechanisms, the result of expanded plasma volume mediated by IL-6, or increased capillary permeability, mediated by both IL-6 and TNF-α. The change is too rapid to be explained by an IL-6 mediated decrease in hepatic synthesis.

The increased SA values associated with 6 months of treatment in nonreactional LL patients, and the rise in HGB values in those not receiving dapsone, may be understood as a consequence of treatment induced amelioration of those cytokines which reduced the SA and HGB values in the first place, IL-1 and TNF-α. This is consistent with the previously reported reduction in serum TNF-α concentration associated with long-term antimicrobial treatment ([29]).

Clinical interpretation. For the physician confronted with a patient having symptomatic ENL of recent onset and a sudden fall in HGB values, attribution of the anemia to ENL, and not the concurrent use of dapsone, can be made with confidence given several considerations. A synchronous fall in SA values would be strong evidence of an ENL-associated condition. Use of dapsone for several months with an incrementally gradual fall in HBG concentration preceding an ENL-associated abrupt fall, would point away from a dapsone effect, toward ENL.

The problem of variation. Taken as an aggregate, patients with ENL do have significant falls in HGB and SA values, and these falls may be understood as the consequence of cytokines which mediate ENL, but it is puzzling that these changes are not found at all in approximately one quarter of the subjects. Conceivable explanations in-

clude masking by dehydration, and differing genotypes for similar phenotypes, but the recognized inherent variation in complex biologic phenomena is particularly attractive in cytokine mediated conditions ([3]). Non-linear cytokine interactions are considered to be extremely sensitive to initial conditions, producing apparently chaotic or nonuniform behavior.

SUMMARY

Changes in hemoglobin (HGB) and serum albumin (SA) concentration associated with the onset of symptomatic erythema nodosum leprosum (ENL) were studied by comparing the values obtained on the day thalidomide or prednisone therapy commenced, with each patients' preceding values. In three groups of ENL patients mean HGB values fell with statistical significance: 1) in 38 patients who had been begun on thalidomide in the decade of the 1990s and who had been receiving dapsone for a minimum of 6 months, mean HGB values fell from 13.19 gm/dl to 12.27 gm/dl, or 7.0%, $p = 6.0 \times 10^{-6}$; 2) in 8 patients who were in the active patient file not overlapping with the preceding group, and who had been on dapsone for a minimum of 6 months, mean HGB values feel from 13.40 gm/dl to 11.96 gm/dl, or 10.7%, $p = 0.0015$; and 3) in 8 patients not overlapping with the preceding groups, who were treated with rifampin and minocylcine or clarithromycin mean HGB values fell from 13.25 gm/dl to 12.48 gm/dl, or 5.8%, $p = 0.0035$. In two groups of ENL patients SA values also fell with statistical significance: 1) in 34 patients who were begun on thalidomide in the decade of the 1990s and who had been on dapsone for a minimum of 6 months, mean SA values fell from 4.14 gm/dl to 3.77 gm/dl, or 8.9%, $p = 1.2 \times 10^{-5}$; and 2) in 10 patients from the active file not overlapping with the preceding group, and who had been on dapsone for a minimum of 6 months, mean SA values fell from 4.45 gm/dl to 4.06 gm/dl, or 8.8%, $p = 0.039$. A brisk fall in HGB values was often accompanied by a fall in SA concentration, and vice versa. Recovery from extreme falls in HGB and SA values was complete in 13 weeks. Recovery occurred in the presence of continued dapsone treatment. The falls could be rapid, occurring too soon to be the result of decreased erythropoiesis or hepatic SA synthesis. This study provides no direct evidence as to the mechanism responsible for the fall in these two parameters, but an interleukin-6 mediated hemodilution is an attractive hypothesis. The ENL-associated fall in HGB values was distinct from dapsone-induced hemolysis and the anemia of chronic disease. The ENL-associated anemia is not a good reason to discontinue dapsone therapy.

RESUMEN

Se midieron los niveles de hemoglobina (HGB) y de albúmina sérica (AS) asociados con la aparición de síntomas de eritema nodoso leproso (ENL). Los valores encontrados al inicio del tratamiento con talidomida o prednisona se compararon con los valores observados en estadíos precedentes. En 3 grupos de pacientes con ENL los cambios en los valores promedio de HGB resultaron estadísticamente significativos: 1) en 38 pacientes que habían comenzado su tratamiento con talidomida en la década de los 1990 y quienes habían recibido dapsona por un mínimo de 6 meses, los valores promedio de HGB cayeron de 13.19 gm/dl a 12.27 gm/dl, ó 7.0%, $p = 6.0 \times 10^{-6}$; 2) en 8 pacientes quienes estuvieron dentro del grupo de pacientes activos sin sobreposición con el grupo precedente, y quienes habían recibido dapsona por un mínimo de 6 meses, los valores promedio de HGB cayeron de 13.40 gm/dl a 11.96 gm/dl, ó 10.7%, $p = 0.0015$; y 3) en 8 pacientes, sin sobreposición con los grupos anteriores, quienes fueron tratados con rifampina y minociclina o claritromicina, los niveles promedio de HGB cayeron de 13.25 gm/dl a 12.48 gm/dl, ó 5.8%, $p = 0.0035$. En dos grupos de pacientes con ENL, los valores promedio de AS también mostraron una caída estadísticamente significativa; 1) en 34 pacientes que habían comenzado su tratamiento con talidomida en la década de los 1990s y quienes habían recibido dapsona durante un mínimo de 6 meses, los valores promedio de AS cayeron de 4.14 gm/dl a 3.77 gm/dl, u 8.9%, $p = 1.2 \times 10^{-5}$; y 2) en 10 pacientes del grupo con la enfermedad activa sin sobreposición con el grupo anterior, que habían recibido dapsona durante un mínimo de 6 meses, los valores promedio de AS cayeron de 4.45 gm/dl a 4.06 gm/dl, u 8.8%, $p = 0.039$. La caída en los valores promedio de HGB a menudo estuvo acompañada por una caída en la concentración de AS, y viceversa. La recuperación de la caída extrema en los valores de HGB y SA fue completa en 13 semanas y ocurrió aun en presencia del tratamiento con dapsona. En ocasiones, las caídas fueron demasiado rápidas como para poderse explicar sobre la base de una disminución en la eritropoyesis o en la síntesis hepática de AS. Aunque el estudio no proporciona evidencias directas sobre el mecanismo responsable de la caída en estos dos parámetros, la hemodilución mediada por interleucina-6 parece ser una hipótesis atractiva. La

caída en los niveles de HGB asociada con ENL fue distinta de la hemólisis inducida por dapsona y de la anemia propia de la enfermedad crónica. La anemia asociada con ENL no es una buena razón para suspender el tratamiento con dapsona.

RÉSUMÉ

Les variations de concentration en hémoglobine [HGB] et en albumine sérique [SA], associées à l'apparition d'érythème noueux lépreux (ENL), ont été étudiées en comparant les valeurs obtenues avant et après le jour où le traitement par la prednisolone ou la thalidomide a été établi. Les valeurs moyennes de [HGB] ont diminué de façon statistiquement significative parmi trois groupes de patients souffrant d'ENL: 1) chez 38 patients avec des commémoratifs de début de traitement à la thalidomide dans les années 90 et de traitement à la dapsone d'au moins 6 mois, la [HGB] moyenne a diminué de 13,19 g/dl à 12,27 g/dl, soit 7,0%, $p = 6{,}0 \times 10^{-6}$; 2) chez 8 patients avec des commémoratifs de traitement à la dapsone d'au moins 6 mois, qui étaient dans le fichier des patients en cours de suivi, indépendant du groupe précédent, la [HGB] moyenne a diminué de 13,40 g/dl à 11,96 g/dl, soit 10,7%, p = 0,0015; et 3) chez 8 patients avec des commémoratifs de traitement à la rifanpicine, minocycline et clarythromycine, qui étaient dans le fichier des patients en cours de suivi, indépendant des groupes précédents, la [HGB] moyenne a diminué de 13,25 g/dl à 12,48 g/dl, soit 5,8%, p = 0,0035. Les valeurs de [SA] ont aussi diminué de façon statistiquement significative parmi deux groupes de patients souffrant d'ENL: 1) chez 34 patients avec des commémoratifs de début de traitement à la thalidomide dans les années 90 et de traitement à la dapsone d'au moins 6 mois, la [SA] moyenne a diminué de 4,14 g/dl à 3,77 g/dl, soit 8,9%, $p = 1{,}2 \times 10^{-5}$; 2) chez 10 patients avec des commémoratifs de traitement à la dapsone d'au moins 6 mois, qui étaient dans le fichier des patients en cours de suivi, indépendant du groupe précédent, la [SA] moyenne a diminué de 4,45 g/dl à 4,06 g/dl, soit 8,8%, p = 0,039. Une chute abrupte de [HGB] était souvent accompagnée d'une chute de [SA], et réciproquement. La récupération des chutes les plus marquées des valeurs de [HGB] et de [SA] était complète en 13 semaines. Cette récupération se déroulait normalement, en particulier concomitamment à un traitement ininterrompu à la dapsone. Ces chutes étaient en général rapides, se produisant trop rapidement pour être la conséquence d'une diminution d'érythropoïèse ou de synthèse hépatique d'albumine. Cette étude n'apporte aucune démonstration directe du mécanisme responsable de la diminution de ces deux paramètres; cependant, une hémodilution secondaire à l'action de l'interleukine 6 est une hypothèse très séduisante. La chute des valeurs de [HGB] était distincte de l'hémolyse induite par la dapsone ou l'anémie associée aux maladies chroniques. L'anémie associée à l'ENL n' est pas une raison suffisante pour interrompre le traitement à la dapsone.

REFERENCES

1. Atkins, M. B., Kappler, K., Mier, J. M., Isaacs, R. E. and Berkman, E. M. Interleukin-6-associated anemia: determination of the underlying mechanism. Blood **86** (1995) 1288–1291.
2. Bharadwaj, V. P., Venkatesan, K., Ramu, G. and Desikan, K. V. Serum iron and total iron binding capacity in leprosy patients. Lep. India **50** (1978) 11–17.
3. Bonn, D. Biocomplexity: look at the whole, not the parts. Lancet **357** (2001) 288.
4. Byrd, S. R. and Gelber, R. H. Effect of dapsone on haemoglobin concentration in patients with leprosy. Lepr. Rev. **62** (1991) 171–178.
5. Davis, D., Charles, P. J., Potter, A., Feldmann, M., Maini, R. N. and Elliot, M. J. Anemia of chronic disease in rheumatoid arthritis: in vivo effects of tumor necrosis factor alpha blockade. Br. J. Rheumatol. **36** (1997) 950–956.
6. Feelders, R. A., Vreugdenhil, G., Eggermont, A. M. M., Kuiper-Kramer, P. A., van Eijk, H. G. and Swaak, A. J. G. Regulation of iron metabolism in the acute-phase response: interferon [gamma] and tumor necrosis factor [alpha] induce hypoferraemia, ferritin production and a decrease in circulating transferrin receptors in cancer patients. Eur. J. Clin. Invest. **28** (1998) 520–527.
7. Fleck, A., Hawker, F., Wallace, P. J., Raines, G., Trotter, J., Ledingham, J. McA. and Calman, K. C. Increased vascular permeability: a major cause of hypoalbuminaemia in disease and injury. Lancet **1** (1985) 781–784.
8. Foss, N. T., de Olivera, E. B. and Silva, C. L. Correlation between TNF production, increased C-reactive protein level and suppression of T-lymphocyte response to concanavalin A during erythema nodosum leprosum. Int. J. Lepr. **61** (1993) 218–226.
9. Grossman, R. M., Krueger, J., Yourish, D., Grane Li-Piperno, A., Murphy, D. P., May, L. T., Kupper, T. S., Sehgal, P. B. and Gottlieb, A. B. Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. PNAS **86** (1989) 6367–6371.
10. Grupta, R. K., Mukheja, R. D., Gupta, S. and Samuel, K. C. Electrophoretic pattern of serum proteins in leprosy. Int. J. Lepr. **44** (1976) 453–455.
11. Hastings, R. C., Trautman, J. R. and Enna, C. D. Thalidomide in the treatment of erythema nodosum leprosum. Clin. Pharmacol. Ther. **11** (1970) 481–487.
12. Hussain, R., Lucas, S. B., Kifayet, A., Jamil, S., Raynes, J., Uqual, Z., Dockrell, H. M., Chiang, T. J. and McAdam, K. P. Clinical and histologic discrepancies in diagnosis of ENL reactions classified by assessment of acute phase proteins SAA and CRP. Int. J. Lepr. **63** (1995) 222–230.
13. Jablons, D. M., Mule, J. J., McIntosh, J. K., Sehgal, P. B., May, L. T., Huang, C. M., Rosenberg, S. A. and Lotze, M. T. IL-6/IFN-beta-2 as a circulating hormone. Induction by cytokine ad-

ministration in humans. J. Immunol. **142** (1989) 1542–1547.

14. Kapoor, K. K., Gupta, S. C., Mehrotta, T. N. and Issac, J. J. Electrophoretic pattern of serum proteins in leprosy. Indian J. Med. Res. **59** (1971) 1424–1429.
15. Launois, P., Vandenbussche, P., M'Bayame, N. N., Drowart, A., van Vooren, J. P., Sarthou, J. L., Millan, J. and Huygen, K. IL-6 production in response to purified mycobacterial heat-shock proteins and to antigen 85 in leprosy. Cell. Immunol. **148** (1993) 283–290.
16. Margarson, M. P. and Soni, N. Serum albumin: touchstone or totem? Anaesthesia **53** (1998) 789–803.
17. McAdam, K. P., Anders, R. F., Smith, S. R., Russell, D. A. and Price, M. A. Association of amyloidosis with erythema nodosum leprosum reactions and recurrent neutrophil leukocytosis in leprosy. Lancet **2** (1975) 572–573.
18. Means, R. T. The anemia of chronic disorders. In: *Wintrobe's Clinical Hematology.* 10th ed. C. R. Lee, F. Paraskevas, J. Forrester, J. P. Greer, J. Lukens, eds. Baltimore: Williams & Wilkins, 1999, pp. 1011–1021.
19. Moraes, M. O., Sarno, E. N., Almeida, A. S., Saraiva, B. C. C., Nery, J. A. C., Martins, R. C. L. and Sampaio, E. P. Cytokine mRNA expression in leprosy: a possible role for interferon-[gamma] and interleukin-12 in reactions (RR and ENL). Scand. J. Immunol. **50** (1999) 541–549.
20. Nieken, J., Mulder, N. H., Buter, J., Vellenga, E., Limburg, P. C., Piers, D. A. and de Vries, E. G. E. Recombinant human interleukin-6 induces a rapid and reversible anemia. Blood **86** (1995) 900–905.
21. Nishimoto, N., Sasai, M., Shima, Y., Nakagawa, M., Matsumoto, T., Shirai, T., Kishimoto, T. and Yoshizaki, K. Improvement in Castleman's disease by humanized anti-interleukin-6 receptor antibody therapy. Blood **95** (2000) 56–61.
22. Ochoa, M. T., Valderrama, L., Ochoa, A., Zea, A., Escobar, C. E., Moreno, L. H. and Falabella, R. Lepromatous and tuberculoid leprosy: clinical presentation and cytokine responses. Int. J. Dermatol. **35** (1996) 786–790.
23. Olencki, T., Finke, J., Tubbs, R., Elson, P., McLain, D., Herzog, P., Budd, G. T., Gunn, H. and Bukowski, R. M. Phase 1 trial of subcutaneous IL-6 in patients with refractory cancer: clinical and biological effects. J. Immunother. **5** (2000) 549–556.
24. Oyama, N., Iwatsuki, K., Homma, Y. and Kaneko, F. Induction of transcription factor AP-2 by inflammatory cytokines in human keratinocytes. J. Invest. Dermatol. **113** (1999) 600–606.
25. Parida, S. K., Grau, G. E., Zaheer, S. A. and Mukherjee, R. Serum tumor necrosis factor and interleukin 1 in leprosy and during leprosy reactions. Clin. Immunol. Immunopathol. **63** (1992) 23–27.
26. Rea, T. H. Frequency and extent of thickening of the nucleated epidermis in leprosy lesions. Int. J. Lepr. **68** (2000) 410–416.
27. Rea, T. H. and Sieling, P. A. Delayed-type hypersensitivity reactions followed by erythema nodosum leprosum. Int. J. Lepr. **66** (1998) 316–327.
28. Ridley, D. S. Histological classification and the immunological spectrum of leprosy. Bull. WHO **51** (1974) 451–465.
29. Sampaio, E. P., Kaplan, G., Miranda. A., Nery, J. A. C., Miguel, C. P., Viana, S. M. and Sarno, N. S. The influence of thalidomide on the clinical and immunological manifestation of erythema nodosum leprosum. J. Infect. Dis. **168** (1993) 408–414.
30. Sampaio, E. P., Moraes, M. O., Nery, J. A. C., Santos, A. R., Matos, H. C. and Sarno, E. N. Pentoxifylline decreases *in vivo* and *in vitro* tumor necrosis factor-alpha (TNF-[alpha]) production in lepromatous leprosy patients with erythema nodosum leprosum (ENL). Clin. Exp. Immunol. **111** (1998) 300–308.
31. Sarno, E. N., Grau, G. E., Vieira, L. M. M. and Nery, J. A. Serum levels of tumor necrosis factor-alpha and interleukin-1 beta during leprosy reactional states. Clin. Exp. Immunol. **84** (1991) 103–108.
32. Sen, R., Yadav, S. S., Singh, V., Sehgal, P. and Dixit, V. D. Patterns of erythropoiesis and anemia in leprosy. Lepr. Rev. **62** (1991) 158–170.
33. Shwe, T., Toe, T. and Tu, A. T. Serum iron and total iron binding capacity in Burmese patients with leprosy. Lepr. Rev. **47** (1976) 287–290.
34. Sosman, J. A., Aronson, F. R., Sznol, M., Atkins, M. B., Dutcher, J. P., Weisi, G. R., Isaacs, R. E., Margolin, K. A., Ernest, M. L., Mier, J., Oleksowicz, L., Eckhardt, J. R., Levitt, D. and Doroshow, J. H. Concurrent phase I trials of intravenous interleukin-6 in solid tumor patients: reversible dose-limiting neurological toxicity. Clin. Cancer Res. **3** (1997) 39–46.
35. Swaak, A. J., Lienard, D., Schraford, T., Koops, K. H., Lejeune, F. J. and Eggermont, A. M. Effects of recombinant tumor necrosis factor (rTNFα) in cancer. Observations on the acute phase protein reaction and immunoglobulin synthesis after high dose recombinant TNF-alpha administration in isolated limb perfusions in cancer patients. Eur. J. Clin. Invest. **23** (1993) 812–818.
36. Wu, C. W., Hsieh, M. C., Lo, S. S., Tsay, S. H., Li, A. F., Lui, W. Y. and P'eng, F. K. Prognostic indicators for survival after curative resection for patients with carcinoma of the stomach. Dig. Dis. Sci. **42** (1997) 1265–1269.
37. Yamamura, M., Wang, X-H., Ohmen, J. D., Uyemura, K., Rea, T. H., Bloom, B. R. and Modlin, R. L. Cytokine patterns of immunologically mediated tissue damage. J. Immunol. **149** (1992) 1470–1475.