Volume 70, Number 3
Printed in the U.S.A.
(ISSN 0148-916X)

INTERNATIONAL JOURNAL OF LEPROSY
and Other Mycobacterial Diseases

VOLUME 70, NUMBER 3 SEPTEMBER 2002

Elevated Platelet Counts and Thrombocytosis in Erythema Nodosum Leprosum[1]

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An interleukin-6 (IL-6) mediated hemodilution was hypothesized to be the likely mechanism for the fall in both hemoglobin and serum albumin values reported in association with erythema nodosum leprosum (ENL) ([25]). Because other ENL-associated phenomena, such as serum amyloid-associated protein and C-reactive protein ([15]), are likely IL-6 mediated ([18]), and, because IL-6 is recognized as an important mediator of reactive thrombocytosis ([1]), it was considered likely that a thrombocytosis, or at least an elevated platelet count, also should be present in patients with ENL. The addition of an automated platelet count to our Complete Blood Count, introduced in 1994, made possible a retrospective testing of this idea, as reported herein.

MATERIALS AND METHODS

All the subjects studied were from those included in the previous report concerning hemoglobin and serum albumin values in ENL, who were started on thalidomide between 1994 and 199, inclusively. Patients were classified according to the criteria and nomenclature of Ridley and his colleagues ([27]). Criteria for the diagnosis of ENL were those previously published ([26]).

Criteria for inclusion were the onset of ENL after 6 or more months of antimicrobial therapy, if dapsone was used, the presence of a recorded platelet count on the day thalidomide was started, and the presence of three platelet counts prior to the onset of ENL, the average of which served as a control or baseline value. Patients receiving prednisone for ENL prior to starting thalidomide were excluded from study. Data for serum albumin and hemoglobin values were also evaluated in a similar manner in the patients selected.

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

The data presented were obtained in the medical center's clinical laboratory as part of routine patient care. Normal values for platelets were 145 to 340 $\times$ 10^3/mm^3.

[1] Received for publication on 4 June 2002. Accepted for publication on 13 August 2002.

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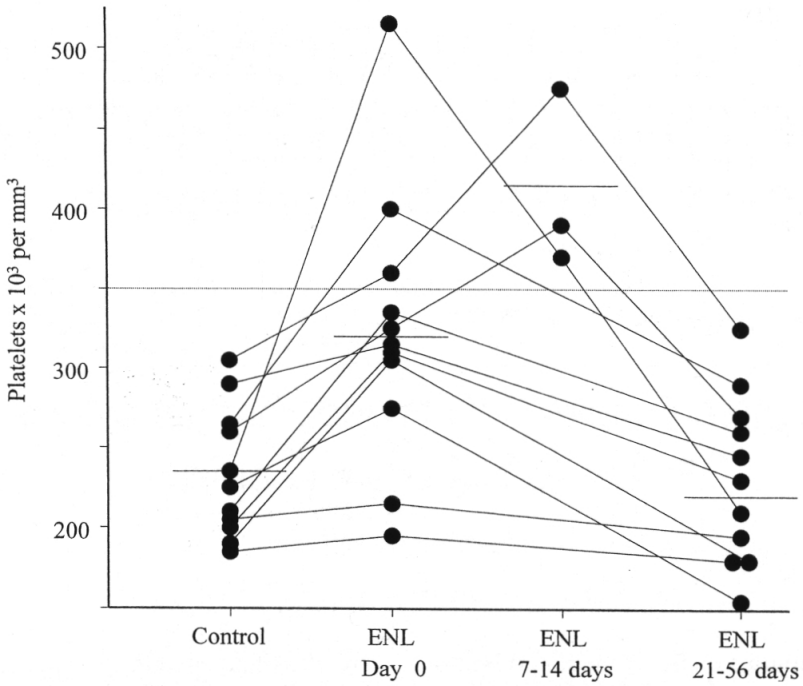

THE FIGURE. Changes in platelet counts in the 11 patients studied. The control values in each patient is the average of the 3 values preceding the onset of symptomatic ENL. "ENL day 0" is the value on the day thalidomide was started. "ENL 7–14 days" is the value obtained after 7–14 days of thalidomide. "ENL 21–56 days" is the first value obtained after 3 weeks of thalidomide therapy. The horizontal solid lines represent mean values. The horizontal dotted line represents the upper limit of normal.

Statistical analysis utilized the Microsoft Excel program, utilizing the "*t*-test: Paired Two Sample for Means." A "p-value" of less than 0.05, using a one-tail test, was considered to be significant.

RESULTS

Eleven patients, 9 Mexican and 2 Filipino, 10 men and 1 woman, met the selection criteria. Their ages ranged from 22 to 64 with a median age of 34. The duration of symptomatic ENL prior to starting thalidomide ranged from 5 to 41 days, with a mean of 13.3 and a median of 14 days.

The control and ENL-associated platelet values are summarized in The Figure. All control values were within normal limits, and, with the onset of ENL, rose appreciably in 9, exceeding the normal upper limit in 3. Mean values increased significantly from a control of 235 to 322 $\times$ $10^3/mm^3$ on the day thalidomide was begun, $p = 0.000237$. In the 3 patients in whom data were available within 1 to 2 weeks of thalidomide therapy, the platelet counts rose further in 2, and decreased in one, but had a mean value of 414 $\times$ $10^3/mm^3$. By 3 to 12 weeks of thalidomide all platelet values were within normal limits, the mean value slightly but insignificantly lower than that of the controls, 233 $\times$ $10^3/mm^3$, $p = 0.27$.

In these same patients, on the day thalidomide was started, mean serum albumin values fell significantly from a control value of 4.3 g/dl to 3.8 g/dl, $p = 0.026$, and hemoglobin values fell insignificantly from a control of 13.2 g/dl to 12.4 g/dl, $p = 0.13$.

The pre-ENL platelet control values were similar to that observed in 11 untreated, non-reactional lepromatous (LL) individuals, means of 235 and 229 $\times$ $10^3/mm^3$, respectively, and medians of 221 and 222 $10^3/mm^3$, respectively.

There was no consistent, proportional relationship between percent change in platelet values and percent changes in either hemoglobin or serum albumin values, i.e., no significant correlations were evident, data not shown.

DISCUSSION

An elevation of the platelet count is a part of untreated, symptomatic ENL. This finding is apparently contrary to that of Hastings, *et al.* [10], who observed a statistically insignificant fall in platelet values in 5 patients with ENL before beginning thalidomide. In particular, these 5 patients differed from those in this study in that the former were receiving anti-inflammatory medication until 3 days before starting thalidomide.

When caused by over-production, two types of pathologic elevations of platelet counts, or thrombocytosis, are recognized, primary or clonal and secondary or reactive [9, 14, 35]. Most examples of clonal thrombocytosis are found in myeloproliferative disorders. Most examples of reactive thrombocytosis (RT) are associated with infection, chronic inflammation, malignancies, or traumatic tissue injury. The elevations observed in ENL, being reversible and occurring in overtly toxic patients, are clearly reactive.

Several lines of evidence support the hypothesis that, of the cytokines known to influence platelet production, IL-6 is of particular importance in mediated RT. Seminal observations have come from animal work, such as the *in vitro* promotion of maturation of murine megakaryocytes by human IL-6 [16], and the *in vivo* production of both peripheral thrombocytosis and megakaryocyte maturation in primates by the administration of recombinant human IL-6 (rhIL-6) [33].

A second line of evidence has been the association of high levels of IL-6 in patients with RT, 60% of 64 patients in one series [35], and 83% of 143 subjects in another [14], but in neither was there a significant correlation between platelet and IL-6 values. Also, in a number of particular diseases, or subsets thereof, an association of RT with high serum IL-6 values has been observed. These include, but are not limited to, Kawasaki disease [17], Hodgkin's disease [3], pleural effusions [38], inflammatory bowel disease [11], and juvenile rheumatoid arthritis [5].

A third line of evidence comes from two conditions, Castleman's disease [23] and malignant mesothelioma [12], where many of the characteristic clinical and laboratory abnormalities, including thrombocytosis, are attributable to endogenous over-production of IL-6. Similarly, in the paraneoplastic syndrome of cholestasis associated with renal-cell carcinoma (Stauffer's syndrome), the high incidence of thrombocytosis, and some other features, as well, appear to be directly related to excessive IL-6 production by the tumor [2].

A fourth line of evidence linking IL-6 to RT is the thrombocytosis found in association with the administration of rhIL-6 to humans with advanced malignancies [7, 22, 24, 36]. In three studies a dose-dependent increase in platelet count was observed [7, 24, 36], and in one a thrombocytosis was noted to be the most consistent hematological change [24]. In all four an anemia of rapid onset was noted, and in one hypoalbuminemia [22].

A fifth line of evidence has come from a blocking study, where monoclonal, humanized anti-IL-6 receptor antibodies were used to treat 7 patients with Castleman's disease [23]. This therapy was associated with decreased platelet counts, as well as increased hemoglobin and serum albumin values. The use of the same antibody to treat patients with refractory rheumatoid arthritis was associated with clinical improvement, falling platelet counts and rising hemoglobin and serum albumin values [39]. An anti-IL-6 antibody used to treat patients with Stauffer's syndrome was associated with lower platelet counts [2].

Further evidence of IL-6 activity comes from the finding of increased levels of acute phase reactants in the human studies cited above, IL-6 being the most potent known stimulant of hepatic production of acute phase reactants [18]. The association is so firm that some authors regard C-reactive protein determination as an economical surrogate for IL-6 measurement [35].

The pathways by which IL-6 may mediate RT are not fully elucidated. *In vitro* data is consistent with a direct effect of IL-6 upon megakaryocytes [16]. More recent studies provide evidence that IL-6 also acts indirectly *in vivo* [4, 11, 17, 19], stimulating thrombopoietin production from the liver, the latter, perhaps, behaving as an acute phase reactant [4].

As a practical matter, the observed thrombocytosis is of little importance. A reactive thrombocytosis, unlike the clonal variety, in and of itself, is not associated with thrombophlebitis, which does not occur un-

less other risk factors are present ([9]). This general rule is in good accord with the experience of this clinic where over 300 patients with ENL, warranting treatment with thalidomide or prednisone, have been managed, but thrombophlebitis has occurred in only 2. Both were in infertile, menstruating women who were unwilling to have tubal ligation, at that time a prerequisite for thalidomide therapy, hence suffering from over two years of high-dose prednisone treatment, before developing thrombophlebitis while on bed rest in a hospital.

However, thrombocytosis is of importance as a phenomenon to be understood in further elucidating the pathogenesis of ENL. Considering the evidence of increased levels of IL-6 in blood from ENL patients ([28, 29]), and increased levels of mRNA coding for IL-6 in ENL lesions ([21, 37]), the RT in ENL is consistent with IL-6 mediation. Two likely origins for increased IL-6 activity in patients with ENL can be postulated, a consequence of TNFα activity, and synthesis in activated neutrophils. TNFα is well known to be elevated in ENL ([28, 29, 31]), and IL-6 is known to rise following infusion of TNFα ([18, 34]). The recent report of the ligation of neutrophil CD44, be it by either antibody or heparin, resulting in the *de novo* synthesis of IL-6 by neutrophils, suggests that the infiltration of neutrophils into ENL lesions, provides an alternative, perhaps TNFα independent, explanation for elevated quantities of IL-6 in ENL ([32]). That RT can occur without an elevation in TNFα, is evident from studies of patients with traumatic tissue injury ([8, 13]). The *in vitro* finding of IL-6 release from leprotic peripheral blood mononuclear cells, upon stimulation with mycobacterial heat-shock proteins suggest yet another mechanism of origin ([20]).

In one study, a significant fall in platelet count occurred on day 3 of rhIL-6 administration, followed by a significant increase by day 7 ([22]), and a parallel change was also reported in primates receiving this cytokine ([33]), observations which reconcile the differences found in ENL patients by Hastings, *et al.* ([10]), and the present study. The early onset of the IL-6-induced increase in plasma volume could easily reduce the concentration of platelets, as it did of hemoglobin and serum albumin ([22]), before the onset of increased platelet production.

Many of the changes following exogenously administered IL-6 suggest that some of the other changes found in ENL could be mediated, at least in part, by IL-6. For example, considering clinical toxicity, all patients developed chills, fever, anorexia, and fatigue. Leukocytosis was inconstant but common. Acute-phase protein changes in common included increasing levels of C-reactive protein, serum amyloid associated protein ([15]), and α-1-antitrypsin ([6]). Other studies support such speculation. For example, the *in vitro* finding of synergism by interferon-gamma of IL-6 synthesis in neutrophils with ligated CD44 ([32]), offers a possible explanation of how the administration of recombinant, human interferon-gamma to patients with lepromatous leprosy might precipitate ENL ([30]).

These speculations concerning IL-6 as a mediator of some of the clinical and laboratory manifestations of ENL are relevant to ideas about its pathogenesis, but must be tempered by several considerations. In particular, cytokines do not act alone, but in networks. In addition, cytokines are pleotropic and redundant, making it difficult to attribute any one phenomenon to any one cytokine with absolute certainty. Also, IL-6 is one of a family of cytokines sharing overlapping activities, a similar tertiary structure, and signaling through a gp120 co-receptor ([1]). However, the production of putative IL-6 effects by the administration of rhIL-6, and of their blocking with either a humanized anti-IL-6 receptor antibody or an anti-IL-6 antibody, offers a high standard of evidence that IL-6 might be important in the pathogenesis of ENL.

SUMMARY

Changes in peripheral blood platelet counts associated with the onset of symptomatic erythema nodosum leprosum (ENL) were studied by comparing, in each patient, the value obtained on the day thalidomide therapy commenced with the average of the three preceding values. In the 11 patients studied, the mean platelet count rose from 235 to 322 × 10^3/mm^3, $p < 0.001$. In 3, the platelet count was above the normal limit, qualifying as thrombocytosis, in 7 the rise was appreciable, and in 2 it was negligible. In the 3 patients studied 1–2 weeks after be-

ginning thalidomide, the mean count was $414 \times 10^3/mm^3$. Counts obtained after 3 or more weeks of thalidomide therapy were within normal limits. This study provided no direct evidence as to the mechanism responsible for the elevated platelet count, but mediation by interleukin-6 (IL-6) was concluded to be an attractive hypothesis, consistent with prior studies of IL-6 in reactive thrombocytosis and of IL-6 in ENL.

RESUMEN

Se estudiaron los cambios en las cuentas de plaquetas circulantes asociados con la aparición de eritema nudoso leproso (ENL). Se compararon las cuentas de plaquetas obtenidas el día de inicio de la terapia con talidomida, con las cuentas promedio de tres determinaciones posteriores. En 11 de los pacientes estudiados, la cuenta promedio de plaquetas aumentó de 235 a $322 \times 10^3/ mm^3$ ($p < 0.001$); en 3 pacientes las cuentas estuvieron por arriba del límite normal y fueron indicativas de trombocitosis, en 7, la elevación fue apreciable, y en 2 fue insignificante. En los 3 pacientes con trombocitosis la cuenta promedio de plaquetas fue de $414 \times 103/mm^3$ después de 1–2 semanas de iniciado el tratamiento con talidomida. Las cuentas obtenidas después de 3 o más semanas de tratamiento estuvieron ya dentro de límites normales. Aunque este estudio no proporciona evidencia directa sobre el mecanismo que conduce a la elevación plaquetaria, se concluyó que la participación de la interleucina 6 (IL-6) es una hipótesis atractiva ya que es congruente con estudios previos sobre la participación de la IL-6 en la trombocitosis reactiva y en el desarrollo de ENL.

RÉSUMÉ

Des modifications de comptages des plaquettes sanguines ont été associées avec l'apparition de l'érythème noueux lépreux (ENL). Elles furent ici étudiées en comparant, pour chaque patient, la valeur obtenue le jour de la mise en œuvre du traitement au thalidomide avec la moyenne des 3 valeurs précédentes. Parmi les 11 patients étudiés, le nombre plaquettaire moyen augmenta de 235 à $322 \times 10^3/mm^3$, $p < 0.001$. Chez 3 patients, le nombre de plaquettes était au dessus de la valeur limite de la variation normale, qualifiant ainsi comme une thrombocytose; chez 7 patients, l'augmentation était assez nette et chez 2, elle était négligeable. Parmi 3 patients étudiés 1 à 2 semaines après la mise en œuvre du traitement à la thalidomide, le nombre plaquettaire moyen était de $414 \times 10^3/mm^3$. Les valeurs plaquettaires obtenues après 3 semaines ou plus suivant la mise en œuvre du traitement étaient dans les limites de la variation normale. Si cette étude n'a pas montré directement de mécanisme expliquant cette élévation du nombre de plaquettes, il fut conclu qu'une médiation par l'interleukine-6 (IL-6) était l'hypothèse la plus séduisante, en accord avec des études précédentes reliant l'IL-6 et les thrombocytoses réactionnelles, ainsi que l'IL-6 dans l'ENL.

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