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Thalidomide—Effect on T Cell Subsets as a Possible Mechanism of Action[1]

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Thalidomide, α-N-pthalimidoglutarimide, was synthesized in 1956 ([11]). First known as a good hypnotic and sedative agent, it was soon withdrawn from the market because of its teratogenic effects ([2]). In 1965, Sheskin, in Israel, first reported the beneficial effects of the drug on the erythema nodosum leprosum (ENL) lepra reaction ([17]). Other investigators throughout the world promptly confirmed Sheskin's findings ([8]) and, ever since, thalidomide has remained the drug of choice for the therapy of ENL. However, its mechanism of action is poorly understood. Initially, ENL was thought to be the result of an immune-complex-mediated mechanism (type III hypersensitivity reaction) ([19]). Thalidomide was thought to act by somehow depressing humoral immunity. However, the effect of thalidomide in immune-complex-mediated diseases has not been proved. The Arthus reaction is not blocked by thalidomide in animal models ([18]). The drug has not been useful in most diseases in which the immunopathogenesis rests on immune-complex-induced damage, such as systemic lupus erythematosus. On the other hand, the idea that ENL is an immune-complex-mediated disease has been largely challenged. Early observations made by Goihman-Yahr, *et al.* ([4–6]) indicated beneficial effects of thalidomide in adjuvant disease of the rat, a condition mediated by delayed hypersensitivity mechanisms and very similar to ENL. Recently, Mshana has supported the same idea ([13]). Furthermore, thalidomide has been useful in conditions such as actinic prurigo ([9]), a non-immune-complex-mediated disorder ([12]).

Attention also has been called to the possibilities that thalidomide may act on other parameters of the immune response, such as cell-mediated immune mechanisms, neutrophilic chemotaxis, phagocytosis, and perhaps even at the level of the nonspecific inflammatory response by opposing the effects of mediators such as prostaglandins, histamine, and 5-OH tryptamine ([1, 7]). All of the postulated mechanisms for the therapeutic effects of thalidomide, however, have been controversial and there is no agreement on the mechanism of action of the drug at the present time.

The number of diseases in which thalidomide has proved to be useful increases every year. Such diseases do not share any apparent common immune aberration capable of pinpointing thalidomide's mechanism of action. For that reason, the drug may be acting in a more basic area of the immune response, i.e., immunoregulation.

Nowadays with the availability of monoclonal antibodies to T cells and their subsets, it is possible to study their proportions in the peripheral blood of patients with several immune-mediated diseases ([15]). Accordingly, we performed the following study.

MATERIALS AND METHODS

Patient selection. Three lepromatous leprosy patients suffering from ENL were given 300 mg per day of thalidomide to control their conditions. The proportion of T cells and their subsets were assessed prior to therapy and one week later.

A 14-year-old girl who fulfilled the standard criteria for the diagnosis of Behçet's syndrome ([3]) was given 200 mg of thalidomide daily after failure to control her disease with prednisone and colchicine. Her peripheral blood T lymphocytes and subsets were measured prior to therapy and 24 hr, 1 week, and 3 months afterward.

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Two patients with actinic prurigo, who were induced into remission by administration of thalidomide, had their T lymphocytes and subsets assessed prior to therapy and one week later.

Cell enumeration. Peripheral blood was obtained from the patients and lymphocytes were separated through a Ficoll-Hypaque gradient; 1×10^6 cells were incubated with the monoclonal antibodies OKT3, OKT4, OKT8, and OKIa which detect total T lymphocytes, helper/inducer, cytotoxic/suppressor, and activated T lymphocytes–macrophages–B cells, respectively. After the cells were washed three times with phosphate buffered saline (PBS), they were incubated with fluorescein-labeled mouse anti-IgG for 30 min. After washing with PBS, fluorescent cells were counted in an American Optical microscope equipped with a vertical illuminator. The percentage of fluorescent cells was recorded for each one of the monoclonal antibodies used, and the OKT4/OKT8 ratio was calculated. B cells were counted using fluorescent antibodies to IgM and IgD.

Statistical analysis was made using the paired *t* test.

RESULTS

T lymphocytes and subsets in lepromatous leprosy patients undergoing ENL. No significant difference was noted in total T lymphocytes or suppressor subsets before or after treatment with thalidomide. T helper cells increased from 22.2% to 28.4%, the difference being significant ($p < 0.05$). Ia+ cells decreased from 10.5% to 7.2% but these differences were not statistically significant (The Table).

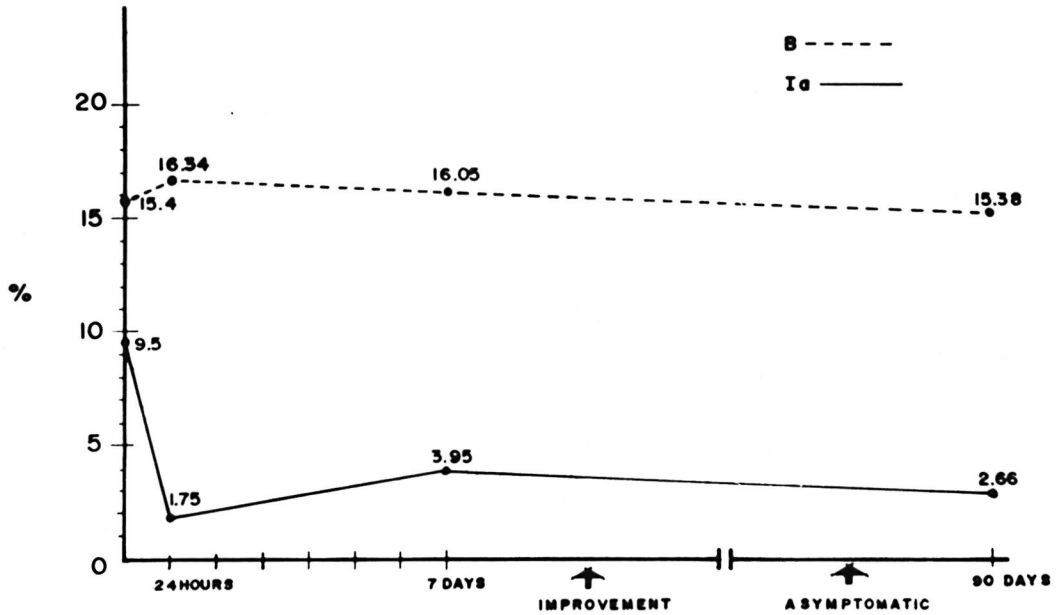
Patient with Behçet's disease. Throughout the three-month course of thalidomide therapy, a persistent decrease in Ia+ cells was observed. The percentage before therapy was 9.5 and fell to 1.75, 3.95, and 2.66 at 24 hr, 1 week, and 3 months, respectively (The Fig.). No apparent variation was noted on the other cell populations.

Both patients with actinic prurigo had their Ia+ cells diminished from baseline levels, but no other changes were observed in the rest of the cell populations examined (The Table).

DISCUSSION

Considering that thalidomide seems to alter T cell populations from patients in our series, it is provocative to think that thalidomide may act primarily by modifying the cells that participate in the immune response. One attractive hypothesis that merits confirmation in a larger survey of cases is that thalidomide may block the action of activated T lymphocytes. It is known that among the several surface markers on T lymphocytes, an Ia antigen becomes apparent only when the cell is activated and not during its resting period ([20]). The anecdotal observation in the girl with Behçet's disease, in whom the proportion of Ia+ cells decreased after thalidomide therapy and remained depressed for the three-month period of therapy, suggests that the possible mechanism of action of thalidomide may be at that level. Of course, it remains to be seen whether the decrease of Ia+ cells in that case is an indication of disease control regardless of the therapeutic agent used to induce the remission. The same findings of a decrease of Ia+ cells was observed following thalidomide therapy in the two patients with actinic prurigo. A tendency toward the same effect in the three lepromatous leprosy patients undergoing ENL was noted, although the difference between pre-therapy and post-therapy values was not statistically significant. This area should be pursued further in a larger series.

Although the use of monoclonal antibodies to analyze lymphoid populations has blossomed widely, this field of immunological research and immune diagnosis is not very well settled. Some lymphoid cells marked with a monoclonal antibody that supposedly defines a certain function, e.g., helper activity, may act to cause other effects, such as in the case of T4+ cells helping to generate suppressor function ([16]). This may be a pertinent although hypothetical explanation for the observation of increased $T4^+$ cells in lepromatous leprosy patients undergoing ENL in this series. It is known that lymphocytes from those patients can generate suppressor function induced by lepromin ([10]). This also would go along with the idea of Mshana ([13]), who feels that ENL is precipitated by an imbalance of T lymphocytes starting with a decrease in sup-



THE FIGURE. B cells and Ia+ cells in a 14-year-old girl with Behçet's disease, three-month course of thalidomide therapy.

pressor T cells. Along the same line, Rea, *et al.* ([14]) found a predominance of suppressor T cells in the skin lesions of lepromatous leprosy patients without ENL.

In conclusion, we feel that our series shows some indication that thalidomide may act by influencing T4 and Ia+ cells, thereby providing some help in a problem of faulty immunoregulation that may underlie the diseases in which thalidomide works therapeutically.

SUMMARY

Thalidomide is the drug of choice in the erythema nodosum leprosum (ENL) type of lepra reaction. Lately it has been used suc-

THE TABLE. *Lymphocyte subsets in peripheral blood from three ENL patients and two actinic prurigo patients (AP). Data presented as mean ± S.D. (range).*

Lymphocyte subset	Baseline		One week after thalidomide	
	ENL	AP	ENL	AP
OKT3+	46.9 ± 4.0% (49.9–41.3)	57.8 ± 3.3% (61.1–54.5)	51.3 ± 7.2% (58.3–41.4)	54.5 ± 3.1% (58.2–51.9)
OKT4+	22.2 ± 3.1% (25.8–18.2)	30.2 ± 1.8% (32.0–28.4)	28.4 ± 5.8%[a] (33.3–20.2)	32.3 ± 5.0% (37.3–27.3)
OKT8+	24.8 ± 2.0% (27.6–23.1)	27.9 ± 1.2% (29.1–26.8)	23.9 ± 1.0% (25.0–22.5)	27.6 ± 1.2% (28.8–26.4)
OKT4+/OKT8+	0.9 ± 0.1 (1.1–0.8)	1.1 ± 0.1 (1.2–1.0)	1.1 ± 0.1 (1.4–1.1)	1.9 ± 0.1 (1.8–2.1)
OKIa+	10.5 ± 5.1% (17.3–5.2)	6.8 ± 2.7% (9.5–4.2)	7.2 ± 0.3%[b] (7.6–7.0)	1.9 ± 0.9% (2.9–1.0)
B cells	ND[c]	10.0 ± 2.0% (12.0–8.0)	ND	12.4 ± 0.4% (12.8–12.0)

[a] $p < 0.05$, paired *t* test, compared to baseline values.
[b] $0.05 < p < 0.10$, paired *t* test, compared to baseline values.
[c] ND = Not done.

cessfully in other diseases, such as discoid lupus erythematosus, actinic prurigo, Behçet's disease, etc. However, its mechanism of action remains unknown.

In patients for whom thalidomide provided relief in their disorder, the proportions of T lymphocytes and their subsets in peripheral blood were assessed by means of monoclonal antibodies.

Three lepromatous leprosy patients with ENL had their T helper populations significantly increased after thalidomide therapy. A 14-year-old girl with Behçet's syndrome showed a consistent decrease in Ia+ cells throughout her three-month course of thalidomide therapy. The same findings were observed in two patients with the actinic prurigo type of polymorphous light eruption. From these results, we conclude that thalidomide may act as an immunomodulating agent on T cell subsets.

RESUMEN

La talidomida es la droga de elección en el tratamiento de la reacción leprosa tipo eritema nodoso leproso (ENL). Ultimamente se ha usado exitosamente en otras enfermedades tales como lupus discoide y eritematoso, prúrigo actínico, enfermedad de Behçet, etc. Sin embargo, su mecanismo de acción permanece desconocido.

Usando anticuerpos monoclonales se cuantificaron las proporciones de linfocitos T y sus subclases en la sangre periférica de pacientes en quienes la talidomida había inducido una clara mejoría.

Tres pacientes lepromatosos con ENL tuvieron elevaciones en sus proporciones de linfocitos T cooperadores después del tratamiento con talidomida. Una niña de 14 años con síndrome de Behçet mostró una consistente disminución en su proporción de células Ia+, durante los 3 meses que duró su tratamiento con talidomida. Los mismos hallazgos se observaron en 2 pacientes con el tipo de prúrigo actínico de la erupción polimórfica ligera. De estos resultados, concluimos que la talidomida puede actuar como un agente inmunomodulador sobre las subclases de células T.

RÉSUMÉ

La thalidomide constitue le médicament de choix dans la réaction lépreuse de type erythème noueux lépreux (ENL). On l'a utilisé récemment avec succès dans d'autres maladies, telles que le lupus erythémateux discoïde, le prurigo actinique, la maladie de Behçet, etc. Le mécanisme de son action demeure cependant inconnu.

Chez les malades auxquels la thalidomide procurait une atténuation de la réaction, on a eu recours à la technique des anticorps monoclonaux pour déterminer les proportions de T lymphocytes et de leurs divers sous-groupes dans le sang périphérique.

Chez 3 malades lépromateux souffrant d'ENL, les populations de cellules "T-helper" ont subi une augmentation significative après le traitement par la thalidomide. Une fille de 14 ans souffrant du syndrome de Behçet a présenté une diminution notable des cellules Ia+ au cours d'un traitement de 3 mois par la thalidomide. Des observations identiques ont été relevées chez 2 malades présentant un prurigo actinique du type d'éruption polymorphe légère. Ces résultats permettent de conclure que la thalidomide peut agir comme agent d'immunomodulation sur les sous-groupes de cellules T.

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