Letter to the Editor

CONCERNS REGARDING **IN VITRO** AND **IN VIVO** USES OF THALIDOMIDE

Since 1965, thalidomide (Thd) has shown dramatic efficacy in alleviating the signs and symptoms of erythema nodosum leprosum (ENL). Despite its well-known teratogenic effects, it is the treatment of choice for acute ENL, with recommended oral doses up to 400 mg daily. The mechanism(s) by which Thd causes deformities in embryos and attenuates ENL, a major cause of morbidity in lepromatous leprosy, are not fully understood. Furthermore, the unknown mechanism of Thd’s therapeutic effect in ENL is accentuated by the uncertainties concerning the factor(s) which cause ENL.

**In vitro** studies to elucidate the mechanism for Thd's therapeutic effect in ENL have focused on evoking cellular synthesis of cytokines and evaluating the effect of Thd on pro-inflammatory cytokines such as TNF-α. An early study reported a selective suppression of TNF-α by Thd, while others reported an enhancement of the synthesis of TNF-α by Thd. These discrepancies in laboratory findings may be due to variations in experimental design such as the concentration of Thd used to treat the cells; the manner in which Thd is prepared; the nature of the stimulant; and the phenotype of the cells (myeloid or lymphoid) stimulated.

As many studies have used concentrations as high as 50 μg/ml to achieve significant suppression of endotoxin-evoked production of TNF-α, there are concerns regarding the concentration of Thd in culture. This concentration of Thd, in our opinion, is far in excess of the effective therapeutic concentrations achieved in treating ENL clinically, and the findings may not be relevant to what occurs **in vivo**. Cells should be exposed to concentrations close to the plasma level of Thd achieved in effective treatment of ENL clinically (i.e. 1–5 μg/ml).

There are concerns about the methods by which Thd is prepared for tissue culture work. Thd is sparingly soluble in aqueous solutions (60 μg/ml in water); it is very susceptible to hydrolysis by hydroxyl ions; and it has a half-life of 8 h in cell culture conditions of 37 °C and 5% CO₂. Hydrolysis of Thd has been shown to abrogate its ability to suppress TNF-α. This suggests that delivery of intact Thd to the responding cell(s) is important for the synthesis of TNF-α. Thd is a non-polar molecule and readily enters into cells. Upon entry, it is probably hydrolyzed via pH dependent spontaneous hydrolysis and/or enzymatic degradation into polar molecules. These polar molecules do not readily exit the cell and accumulate in the cell. Whether or not Thd or one of the numerous hydrolysis products is the effector molecule that suppresses the synthesis of TNF-α, remains to be determined. To minimize variability of results within and between laboratories, we suggest a consistent method of preparation of Thd that reduces hydrolysis, such as use of a non-aqueous solvent such as DMSO, or a solvent more physiological like normal saline solution at pH 3.0.

Concerns exist regarding the nature of the stimulant and the phenotype of the cells stimulated. Thd suppresses TNF-α in T-cell independent systems such as LPS-stimulated monocytes, whereas in T-cell dependent systems such as peripheral blood mononuclear cells stimulated by cross-linking the T-cell receptor, Thd can enhance the production of TNF-α. This enhancement is seen at late stages of incubation (48–72h) and is thought to be dependent on IL-2 signaling. Thd has been shown to enhance IL-2 production, especially by CD4+ cells. The phenotype of the population of cells stimulated should be characterized as much as possible; the stimulant and the conditions of stimulation should be described.
In vivo, Thd has been shown to modulate TNF-α but it does so in a confusing and contradictory manner. In blind or open clinical trials, depending on the clinical condition treated with Thd, improvement may be associated with a decrease of TNF-α in the serum (e.g. ENL patient serum), or with an increase in TNF-α (e.g. aphthous ulcers). In a protocol in which Thd was used to treat toxic epidermal necrolysis, patients receiving Thd were taken off the protocol because of an increase in mortality associated with an increase in TNF-α. In animal studies in which mice were treated with Thd and received lethal doses of endotoxin to induce septic shock, Thd-treated mice were protected, whereas, in another study, Thd-treated mice had a mortality rate greater than the control mice. In a short open clinical study comparing the effects of Thd, pentoxifylline and prednisone in the treatment of ENL, the authors concluded that, in addition to TNF-α, other inflammatory cytokines may be targeted by Thd. To us, the most incriminating evidence that TNF-α is not the sole cytokine targeted by Thd in ENL is the observation that Thd is not an effective treatment for reversal reaction (RR). The pathology of RR, even more so than in ENL, is associated with an increase in TNF-α protein and TNF-α mRNA in the skin and peripheral nerves. Recently, in a study involving leprosy patients experiencing RR, but not treated with steroids, we observed that Thd enhanced TNF-α production and TNF-α mRNA expression when peripheral blood mononuclear cells were stimulated with integral Mycobacterium leprae (unpublished observations).

The growing number of therapeutic applications for Thd in conditions other than ENL, such as refractory multiple myeloma, is promoting more prescription use of Thd. Clinicians should be aware that although Thd generally suppresses TNF-α, it can, under certain circumstances, enhance the synthesis of this pro-inflammatory cytokine. It is generally known among Hansenologists (leprologists) that Thd is an effective treatment for ENL, but not for RR. With the current push towards the integration of leprosy care into the general health system, inexperienced physicians may prescribe Thd for the treatment of RR. Based upon our recent in vitro findings among some RR patients, Thd may exacerbate this condition.

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