Effect of thalidomide on induction of antibody synthesis in mice to the T-independent antigen, DNP-Ficoll

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Summary Thalidomide enhances *de novo* IgM antibody synthesis in mice to DNP-Ficoll. The immunocompetent cells responsive to thymic independent antigens like DNP-Ficoll are macrophages and the B lymphocytes. Enhancement of immunoresponsiveness to DNP-Ficoll seems to be due to augmentation of macrophage function by thalidomide.

Introduction

Since 1965, thalidomide has been used in alleviating the signs and symptoms of a major medical complication of lepromatous leprosy, erythema nodosum leprosum (ENL).¹ Histologically, the hallmarks of ENL lesions are vasculitis or vascular necrosis, oedema and inflammation with infiltrates of neutrophils affecting the entire dermis and subcutaneous fat.² The factor or factors that trigger the influx of neutrophils is unknown. For thalidomide to be effective in ENL, it must interfere with one or more of the essential steps in the pathogenesis of this syndrome.

Since it has been suggested that ENL is an immune complex mediated disease,^{3,4} we have investigated the effect of thalidomide on humoral immunological responses. Thalidomide significantly inhibits an IgM but not an IgG response in mice to the T-cell-dependent antigen, sheep erythrocytes; it also selectively decreases serum IgM concentrations among leprosy patients being treated for their ENL.⁵ These observations have prompted us to speculate that a clinically relevant site of action for thalidomide in ENL is on the synthesis of IgM antibody. If thalidomide acts on immunocompetent cells, the cell target of the drug must be among the macrophage, IgM antibody forming B cell and helper or suppressor lymphocytes.

To exclude T cell interaction and to determine the effect of thalidomide on cell interactions of the macrophage and B cell type, we investigated the effect of thalidomide on IgM responsiveness of mice to DNP-Ficoll. B cell responsiveness to this nitrophenol conjugated polysaccharide is not dependent on T cell help but has been shown to require a phagocytic accessory and B cell interaction.⁶ The accessory cells are adherent, phagocytic and must be viable.

Materials and methods

Animals. Locally bred female Swiss-Webster mice 8–12 weeks of age were used. Thalidomide (Grunenthal GMBH, 5190 Stolberg/RhLd., Federal Republic of Germany) was incorporated in a 0.03% w/w basis in powdered Rodent Lab Chow (Ralston Purina Co., St Louis, MO).

DNP⁴⁰-AECM-Ficoll (Biosearch, San Rafael, CA) was resuspended in sterile saline and injected intravenously into mice. Picrylsulphonic acid, 2,4,6-trinitrobenzinesulphonic acid (TNBS) was purchased from Sigma Chemical Co., St Louis, MO, and conjugated to sheep erythrocytes (SRBC) (Microbiological Associates, Bethesda, MD) to produce trinitrophenol (TNP) substituted cells as described in an earlier study.⁷

Enumeration of plaque-forming cells (PFC). TNP substituted sheep erythrocytes were plated with mouse spleen cells as described previously, for enumeration of SRBC-plaque-forming cells (PFC).⁵ Plaques were corrected for background PFC to SRBC and the contribution of myeloperoxidase positive cells (monocytes-macrophages). Results are expressed as PFC/million peroxidase negative cells. Statistical analysis was done using a Student's *t*-test.

Results

A preliminary experiment was to determine the dose of DNP⁴⁰-Ficoll in mice which would result in 50% of the maximum PFC response to TNP substituted SRBC. Classically at the median effective dose (ED₅₀), a dose response curve is steepest, and this is therefore the area at which inhibition or enhancing phenomena resulting from a drug treatment would be most readily demonstrable. The ED₅₀ for DNP⁴⁰-Ficoll induction of a 4 day direct PFC response was interpolated to be 10^{3.5} ng (Figure 1). To assess the effect of thalidomide on the responsiveness of mice to DNP⁴⁰-Ficoll, mice were fed thalidomide at 0.03% w/w in powdered rodent chow for 7 days. Control mice received no thalidomide. (Thalidomide fed at this concentration for 3 days achieves a mean blood level of intact drug in mice of 0.84 µg/ml⁵, and this is essentially equivalent to that of 0.9 µg/ml achieved in humans following 100 mg oral doses.)⁸ After 7 days, both groups of mice were injected with an ED₅₀ dose of 10^{3.5} ng of DNP⁴⁰-Ficoll. Four

Figure 1. Four day direct PFC (mean $\pm$ SD) of 4 mice per group to DNP⁴⁰-Ficoll.

Figure 2. The effect of thalidomide on 4 day PFC (mean $\pm$ SD) of mice to an ED₅₀ dose (10^{3.5} ng) of DNP⁴⁰-Ficoll.

days later, spleens from both groups of mice were enumerated for their PFC to TNP substituted SRBC. As may be seen in Figure 2, thalidomide enhanced the PFC responsiveness of mice to DNP⁴⁰-Ficoll.

Discussion

The pathogenesis of ENL may be viewed as an immunological puzzle. If the mechanism of action of thalidomide in inhibiting immunological and inflammatory events were known, it would facilitate an understanding of the immunopathology of ENL. We,⁵ and others,⁹ have shown thalidomide to be effective in suppressing a primary humoral response to sheep erythrocytes. The development of optimal antibody responses to T-cell-dependent antigens like SRBC requires the participation of and interactions among at least 3 distinct types of cells in the immune response: macrophages; thymus-derived cells (T cells); and precursors of antibody-producing cells (B cells).

Macrophages have the critical function of presenting antigen in a highly immunogenic form to T and B cells to initiate the immune response.¹⁰ As tested by reticuloendothelial system clearance of colloidal carbon in mice, thalidomide does not decrease macrophage phagocytic capability.⁵ On the contrary, a significantly enhanced phagocytic index over controls was observed in animals fed 0.03% w/w thalidomide for 7 days. In this study, mice were fed thalidomide under similar conditions of time and dose. The immunoreactive cells in focus using the T-independent antigen DNP⁴⁰-Ficoll, are the macrophage and the B cell. Since immunoresponsiveness was enhanced, the function of either the macrophage or B cell (or both) was enhanced by thalidomide. In view of thalidomide's increasing RES clearance of colloidal carbon in mice, and its reported enhancement of monocyte function in a lepromatous patient,¹¹ an augmentation of macrophage function by thalidomide could be an explanation for the enhanced PFC response to DNP⁴⁰-Ficoll and the reduced PFC responses to SRBC. Thalidomide has been shown to stabilize the membranes of human and rat liver lysosomes.¹² By stabilizing membranes, the drug could retard the release of antigens from phagolysosomes. Retention of antigen within phagolysosomes could result in enhanced enzymatic degradation. Antigens, especially proteins on SRBC, could be degraded to less immunogenic moieties. Polysaccharide carrier conjugates like DNP⁴⁰-Ficoll which are more difficult to degrade could be more efficiently processed and presented to B cells.

The enhanced IgM antibody responsiveness to DNP⁴⁰-Ficoll would seem to exclude the B lymphocytes as a target site for thalidomide's effect on the humoral immune responses. The target site for thalidomide among the macrophages and T lymphocytes remains to be elucidated. Thalidomide has been shown to decrease T helper cells in the peripheral blood of healthy males.¹³ Thus thalidomide could inhibit IgM antibody responses to T helper cell dependent antigens like SRBC or possibly *M. leprae* by inhibiting T helper cell activity.

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