Immunomodulation by thalidomide and thalidomide analogues

Laura G Corral, Gilla Kaplan

Tumour necrosis factor α (TNFα), a key cytokine involved in the host immune response, also contributes to the pathogenesis of both infectious and autoimmune diseases. To ameliorate the pathology resulting from TNFα in these clinical settings, strategies for the inhibition of this cytokine have been developed. Our previous work has shown that the drug thalidomide is a partial inhibitor of TNFα production in vivo. For example, when leprosy patients suffering from erythema nodosum leprosum (ENL) are treated with thalidomide, the increased serum TNFα concentrations characteristic of this syndrome are reduced, with a concomitant improvement in clinical symptoms. Similarly, we have found that in patients with tuberculosis, with or without HIV infection, short-term thalidomide treatment reduces plasma TNFα levels in association with an accelerated weight gain. In vitro, we have also shown that thalidomide partially inhibits TNFα produced by human peripheral blood mononuclear cells (PBMC) responding to stimulation with lipopolysaccharide (LPS). Recently, we found that thalidomide can also act as a co-stimulatory signal for T-cell activation in vitro resulting in increased production of interleukin 2 (IL2) and interferon γ (IFNγ). We also observed a bidirectional effect on IL12 production: IL12 production is inhibited by thalidomide when PBMC are stimulated with LPS, however, IL12 production is increased in the presence of the drug when cells are stimulated via the T cell receptor. The latter effect is associated with upregulation of T cell CD40 ligand (CD40L) expression. Thus, in addition to its monocyte inhibitory activity, thalidomide exerts a co-stimulatory or adjuvant effect on T-cell responses. This combination of effects may contribute to the immunomodulating properties of the drug.

To obtain drugs with increased anti-TNFα activity that have reduced or absent toxicities, novel TNFα inhibitors were designed utilizing thalidomide as template. These thalidomide analogues were found to be up to 50 000 times more active than thalidomide. The compounds comprise two different types of TNFα inhibitors. One class of compounds, shown to be potent phosphodiesterase 4 (PDE4) inhibitors, are selective TNFα inhibitors in LPS stimulated PBMC and have either no effect or a suppressive effect on T cell activation. The other class of compounds also inhibit TNFα production, but do not inhibit PDE4 enzyme. These compounds are also potent inhibitors of several LPS induced monocyte inflammatory cytokines. Also, the latter compounds markedly stimulate the anti-inflammatory cytokine IL10. Similarly to thalidomide, these drugs that do not inhibit PDE4 act as costimulators of T cells but are much more potent than the parent drug. The distinct immunomodulatory activity of these new TNFα inhibitors may potentially allow them to be used in the clinic for the treatment of a wide variety of immunopathological disorders of different aetiologies.

TNFα is a key player in the immune response

TNFα is a pleiotropic cytokine produced primarily by monocytes and macrophages, but also by lymphocytes and NK cells. TNFα plays a central part in the host immune response to viral, parasitic, fungal and bacterial infections. The importance of TNFα and TNFα signalling through its receptors in the host immune response to disease has become clearer as a result of a number of seminal studies. For example, mice genetically deficient in TNFα have a significantly reduced humoral immune response to adenovirus infection. In Leishmania major infection, TNFα signalling is important for protection as mice lacking TNFα p55 receptor (TNFR-p55) show delayed elimination of the parasites compared with controls and the lesions formed failed to resolve. Mice deficient in TNFR-p55 are also significantly impaired in their ability to clear infection with Candida albicans and readily succumb to the infection. TNFα signalling is also crucial in resisting Streptococcus pneumoniae infections in mice. In addition, TNFα is essential for protection against murine tuberculosis. TNFR-p55 deficient mice have been shown to be more susceptible to tuberculosis infection. When TNFα was neutralised in vivo by monoclonal antibodies impaired protection against mycobacterial infection was observed. The data from both models also established that TNFα and the TNFR- p55 are essential for production of reactive nitrogen intermediates by macrophages early in infection.

TNFα contributes to disease pathogenesis

Although TNFα is crucial to the protective immune response, it also plays a part in the pathogenesis of both infectious and autoimmune diseases. Increased concentrations of TNFα have been shown to trigger the lethal effects of septic shock syndrome. TNFα has also been implicated in the development of cachexia, the state of malnutrition that complicates the course of chronic infections and many cancers. In rheumatoid arthritis, TNFα is a critical mediator of joint inflammation and therefore an important therapeutic target.
Recently, it has been shown that treatment of patients with neutralising anti-TNFα antibodies produces a dramatic reduction in disease activity in this condition. Similarly, it has been shown that TNFα reduces the production of interferon-gamma (IFN-γ) by CD4+ T cells. TNFα production by CD4+ T cells is regulated by the balance between pro-inflammatory cytokines (e.g., TNFα, IFN-γ) and anti-inflammatory cytokines (e.g., IL-10, IL-4). Inhibition of TNFα production by CD4+ T cells reduces the production of IFN-γ and increases the production of IL-10, leading to a shift in the cytokine profile towards an anti-inflammatory response. Therefore, the inhibition of TNFα production by CD4+ T cells may be a promising target for the treatment of inflammatory diseases.

Thalidomide inhibits TNFα production by monocytes

The pathology associated with TNFα production is profound and in many diseases leads to significant morbidity and mortality. This has led to a concerted effort to discover drugs that will down regulate the production of this cytokine. Agents conventionally used in these diseases may inhibit TNFα production, but are also often broadly immunosuppressive (for example, cyclosporin A and corticosteroids) and therefore associated with extensive side effects. Drugs that are potentially more specific in inhibiting TNFα are under active investigation and development. Our previous work has shown that the drug thalidomide (α-N-phthalimidoglutarimide) is a relatively selective inhibitor of TNFα production by human monocytes in vitro. This property of thalidomide was first described in leprosy patients with ENL, an acute inflammatory complication of lepromatous leprosy that is accompanied by increased serum TNFα levels. Thalidomide treatment of patients with ENL was found to induce a prompt reduction of TNFα serum levels with a concomitant abrogation of clinical symptoms. Furthermore, in patients with tuberculosis, with or without concomitant HIV infection, thalidomide treatment was found to both decrease plasma TNFα protein levels as well as monocyte TNFα mRNA levels. This decrease was associated with an accelerated weight gain. Thalidomide treatment was found to both decrease plasma TNFα protein levels as well as monocyte TNFα mRNA levels. This decrease was associated with an accelerated weight gain. In a rabbit model of mycobacterial meningitis, thalidomide treatment combined with antibiotics produced a marked reduction in TNFα levels, leucocytosis, and brain disease. In addition, thalidomide inhibited TNFα serum levels in mice challenged with LPS, thus partially protecting the animals from septic shock.

In vitro, we have found that thalidomide selectively reduces the production of TNFα by human monocytes cultured in the presence of both LPS and mycobacterial products. However, this inhibition was only partial (50% to 70%) possibly because of the instability of the drug in aqueous solutions. The mechanism by which thalidomide reduces TNFα production is still unclear. The drug seems to inhibit TNFα production by human monocytes in vitro in association with enhanced degradation of TNFα mRNA. It also inhibits the activation of the nuclear factor κB (NFκB), a promoter for the transcription of TNFα as well as transcription of HIV-1.
tial for the survival of CD8+ T cells and that in its absence these cells die or become anergic.30

These studies show that in addition to its inhibitory effect on the production of mono-
cyte cytokines, thalidomide exerts a costimulator-
tory or adjuvant effect on T cell responses. The
immune modulating effects of the drug in
patients may thus be attributable to a balance
between the inhibition of production of mono-
cyte cytokines, including TNFα, and the
co-stimulation of T cell activity. The effects
of thalidomide in vivo in HIV infected patients
seem to reflect the co-stimulatory activity of
the drug.26 In a placebo controlled study to evalu-
ate the effects of in vivo immunomodulation
with thalidomide, the drug was administered
for four weeks to HIV infected patients. Thalidomide treatment did not affect TNFα
levels in these patients. In contrast, thalidomide
treatment resulted in significant immune
stimulation. This was reflected by increases in
DTH responses and increased plasma levels of
T cell activation markers such as soluble IL2
receptor (sIL2R) and soluble CD8 antigen. An
earlier study of tuberculosis patients treated
with thalidomide showed increased plasma
levels of IFNγ suggesting an immunostimula-
tory effect of the drug.31 Recently, patients suf-
ferring from sarcoidosis have shown consistent
increases in sIL2R plasma levels after thalido-
mide treatment (Oliver et al, manuscript in
preparation). In the same study, thalidomide
treatment increased the proliferation of sarco-
id patient T cells in response to concanavalin A
in vitro. These results strongly suggest that
thalidomide directly stimulates T cells in vivo
in patients, corresponding to the T cell
costimulatory properties of the drug observed
in vitro in T cells from normal donors,23 25 as
well as in the T cells of HIV infected patients.26

Thalidomide analogues are improved
TNFα inhibitors
In addition to being the drug of choice for the
treatment of ENL, thalidomide has been
shown to be useful in a number of clinical situ-
ations including rheumatoid arthritis, HIV
associated aphthous ulcers and chronic graft
versus host disease.31–34 However, thalidomide
is a potent teratogen and ingestion of the drug
by a pregnant woman can lead to catastrophic
birth defects.35 In addition, thalidomide treat-
ment is often accompanied by a number of side
effects, including peripheral neuropathy.36

Therefore, the use of thalidomide requires
strict monitoring of all patients.37 Thus, there is
a pressing need to develop drugs with increased
TNFα inhibitory activity and reduced or
absent toxicities. Towards this end, structural
analogues of thalidomide have been designed
and synthesised at Celgene Corporation (War-
ren, New Jersey) and screened for inhibition of
TNFα production. A large number of potent
novel TNFα inhibitors were thus identified.

Recently, some of these compounds were
described.38–40 On a molar basis, the more
potent of these thalidomide analogues were
found to be up to 50 000-fold more potent
than thalidomide at inhibiting TNFα produc-
tion by human PBMC stimulated by LPS in
vitro. Furthermore, we have shown that some
of these compounds retain high activity in LPS
stimulated human whole blood.40 In vivo,
several of these new compounds showed
improved activity in reducing LPS induced
TNFα levels in mice39 and in inhibiting the
development of adjuvant arthritis in rats.40

Thalidomide analogues comprise two
distinct classes of molecules
A group of thalidomide analogues, selected for
their capacity to potently inhibit TNFα pro-
duction by LPS stimulated PBMC, was further
investigated (fig 1). When tested for their effect
in vitro on LPS induced cytokines, different
patterns of cytokine modulation were shown.25

One class of compounds, class I or ImiDs
(Immunomodulatory Imide Drugs) showed
not only potent inhibition of TNFα but also
marked inhibition of LPS induced monocyte
IL1β and IL12 production. LPS induced IL6
was also inhibited by these drugs, albeit
partially. These drugs were potent stimulators
of LPS induced IL10, increasing IL10 levels by
200–300%. In contrast, the other class of com-
ounds, class II or SelCiDs (Selective Cytokine
Inhibitory Drugs), while still potently inhibiting
TNFα production, had a more modest
inhibitory effect on LPS induced IL1β and
IL12, and did not inhibit IL6 even at high drug
concentrations. In addition, SelCiDs produced
a more modest IL10 stimulation (20–50%
increases). In all of these characteristics, SelCi-
dDs were more similar to thalidomide than
ImiDs.16 17

Further characterisation of the SelCiDs
showed that they are potent PDE4 inhibitors.10
PDE4 is one of the major phosphodiesterase
isoenzymes found in human myeloid and lym-
phoid lineage cells.41 The enzyme plays a
crucial part in regulating cellular activity by
degrading the ubiquitous second messenger
cAMP and maintaining it at low intracellular
levels. Inhibition of PDE4 results in increased
cAMP levels leading to the modulation of LPS
induced cytokines including inhibition of
TNFα.42 Increasing intracellular cAMP levels
have been shown to inhibit TNFα production
in monocytes as well as in lymphocytes.41 43
although it is not clear how this inhibition is

Figure 1 Chemical structures of thalidomide and selected thalidomide analogues.
regulated. Interestingly, the IMiDs and thalidomide were found not to inhibit PDE4.

In addition to the differential modulation of LPS induced monocyte cytokines, the two classes of compounds showed distinct effects on T cell activation. SelCIDs, the non-PDE4 inhibitors, had little effect on T cell activation causing only a slight inhibition of T cell proliferation. This effect was not unexpected as it is well established that increasing cAMP levels in T cells during the early phase of mitogen or antigen activation results in a decrease in proliferative potential. On the other hand, IMiDs, the non-PDE4 inhibitors, were potent costimulators of T cells and increased cell proliferation dramatically in a dose dependent manner. Similarly to thalidomide, these compounds had a greater costimulatory effect on the CD8+ T cell subset than on the CD4+ T cell subset (Corral et al, unpublished observation). IMiDs, when added to anti-CD3 stimulated T cells, also caused marked increases in the secretion of IL2 and IFNγ and induced the up-regulation of CD40L expression on T cells. These findings show that in addition to their strong anti-inflammatory properties, IMiDs efficiently costimulate T cells with 100 to 1000 times the potency of the parent drug. The molecular target of these co-stimulatory cytokine modulating drugs is as yet unknown.

**Thalidomide and IMiDs modulate cytokines differently according to cell type and stimulation pathway**

As described above, thalidomide has been shown to inhibit IL12 production by LPS stimulated monocytes in vitro. In vivo, however, thalidomide treatment of HIV infected and *M tuberculosis* infected patients induced increases in plasma IL12 levels (Bekker et al, submitted data). Thalidomide treatment also resulted in increases in plasma IL12 levels in patients with scleroderma and sarcoidosis (Oliver et al, manuscripts in preparation). These dual and opposite effects of thalidomide may be explained by the differential modulation of cytokines according to target cell type and specific pathways of cellular stimulation. IL12 is produced primarily by APC (monocytes/macrophages and dendritic cells) and is regulated by both T cell dependent and T cell independent pathways. LPS directly induces T cell independent IL12 production by APC, which is inhibited by thalidomide. In the T cell dependent pathway, on the other hand, the production of IL12 by the APC is induced primarily by the interaction of CD40 on the surface of the APC with CD40L on the surface of activated T cells. When T cells were stimulated by anti-CD3, thalidomide and IMiDs treatment caused a significant stimulation of IL12 production. Thalidomide and IMiDs also induced an up-regulation of CD40L on the surface of T cells. Blockade of this pathway inhibits the production of IL12 and abolishes the stimulatory effect of thalidomide. Interestingly, in HIV infected patients, the consistent increases in plasma IL12 levels induced by thalidomide treatment lagged behind the increases in T cell activation markers. This observation suggested that IL12 production was augmented as a consequence of drug induced T cell activation.

The dichotomous nature of thalidomide cytokine modulation may explain the seemingly opposite effects observed in different clinical situations. When patients with Behçet’s syndrome are treated with thalidomide, healing of inflammatory aphthous ulcers occurs, but is sometimes accompanied by exacerbation of erythema nodosum. Similarly, the paradoxical worsening of graft versus host disease and toxic epidermal necrolysis reported in clinical trials of thalidomide may be a manifestation of the unsuspected immune stimulatory effect of this drug.

**Potential clinical applications of thalidomide and thalidomide analogues**

The thalidomide analogues discussed here seem to have retained different properties of the parent drug (table 1). The distinct immunomodulatory activities of these two classes of drugs suggest they may have applications in different immunopathological disorders. SelCIDs, which inhibit PDE4, may be used in clinical situations in which PDE4 inhibition and selective TNFα inhibition are beneficial. Therapeutic increase of intracellular cAMP levels by PDE4 inhibitors has anti-inflammatory effects, which may afford consequent benefits in a variety of diseases such as asthma, atopic dermatitis and rheumatoid arthritis. Indeed, in an animal model of adjuvant arthritis, thalidomide derived PDE4 inhibitors have shown efficacy in suppressing the development of disease as measured by ankle swelling, hind limb radiographic changes and weight gain. The suppression of arthritis was accompanied by a reduction in TNFα and IL2 mRNA levels in the ankle joints of treated rats.

**Table 1 Immunomodulatory profiles of thalidomide and thalidomide analogues**

<table>
<thead>
<tr>
<th>Thalidomide</th>
<th>IMiDs</th>
<th>SelCIDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibits LPS induced inflammatory cytokines TNFα and IL12</td>
<td>Strongly inhibit LPS induced inflammatory cytokines: TNFα, IL1β, IL6 and IL12</td>
<td>Strongly inhibit LPS induced inflammatory cytokines TNFα and IL12</td>
</tr>
<tr>
<td>Stimulates LPS induced anti-inflammatory cytokine IL10</td>
<td>Strongly stimulate LPS induced anti-inflammatory cytokine IL10</td>
<td>Stimulate LPS induced anti-inflammatory cytokine IL10</td>
</tr>
<tr>
<td>Costimulates T cell activation</td>
<td>Strongly costimulate T cell activation</td>
<td>Inhibit or have no effect on T cell activation</td>
</tr>
<tr>
<td>Does not inhibit PDE4</td>
<td>Do not inhibit PDE4</td>
<td>Strongly inhibit PDE4</td>
</tr>
</tbody>
</table>
Other known selective PDE4 inhibitors, such as rolipram, have been reported to have dose limiting side effects, such as nausea and vomiting, which limit the therapeutic use of these drugs. These side effects may be produced by the lack of specificity of these drugs—that is, the compounds inhibit one or more PDE isoenzymes in non-target tissues. For example, it is probable that the emetic activity of PDE4 inhibitors is attributable to an action of the drugs in the CNS. Intensive effort is being directed towards identifying compounds with improved therapeutical ratios. Preliminary results with thalidomide derived PDE inhibitors indicate that these novel drugs are selective inhibitors of PDE4 and may be better tolerated than other PDE4 inhibitors, as they have not shown evidence of emesis in animals. One of these drugs has been recently shown to be well tolerated in a small human safety trial in the United Kingdom (D Stirling, personal communication).

The IMiDS, as thalidomide, are anti-inflammatory drugs that do not target PDE4. These compounds, in addition to their potential use to decrease inflammation, could also be useful in clinical settings where there is a defect in T cell function, as in HIV disease. HIV infection is accompanied by deficiencies in the production of IL12 and in the up-regulation of CD40L. IL12 has been shown to restore HIV specific cell mediated immunity in vitro and to increase HIV specific CTL responses in vivo. Also, deficient IL12 responses in HIV infected patients can be restored in vitro by CD40L and IFNγ, the same costimulatory factors induced by thalidomide and IMiDs. Thus, these drugs may eventually be used to restore or stimulate IL12 production in immune deficient patients.

IL12 has also been shown to exhibit potent anti-tumor activity in murine tumor models through various mechanisms including the stimulation of natural killer cell activity, activation of CD8+ cytotoxic T cells and increased IFNγ mediated anti-angiogenesis. Thalidomide has also recently been reported to exhibit anti-tumor activity through the inhibition of angiogenesis in vivo. However, this anti-angiogenic effect does not seem to be mediated by TNFα inhibition. Although these studies did not determine the mechanism of thalidomide’s anti-angiogenic activity, it is conceivable that stimulation of IFNγ/IL12 levels may be at least partly responsible. One report indicates that thalidomide may have anti-angiogenic activity in multiple myeloma in humans. In summary, our recent findings that thalidomide and IMiDs preferentially costimulate CD8+ T cell and induce T cell dependent IL12 production suggest possible applications of these drugs in the control of viral infections or in boosting anti-tumor immunity. Also, there are anecdotal reports of the efficacy of thalidomide in treating refractory inflammatory bowel disease. Recently, preliminary findings were announced from a pilot study with patients with Crohn’s disease refractory to standard treatments (Annual Digestive Disease Meeting, May 1999, Orlando, FL). In this study, two third of the patients experienced a significant improvement in their condition. This therapeutic effect may be a combination of TNFα inhibition and CD8+ T cell stimulation.

**Conclusions**

In several disease conditions such as septic shock, chronic infections and cancer, overproduction of TNFα is accompanied by severe toxicities. Thalidomide inhibits TNFα production in different diseases without causing the immunosuppression often associated with standard agents such as glucocorticoids and cyclosporin A. Our results indicate that the immunomodulating effects of thalidomide may occur via the inhibition of TNFα production and/or the stimulation of T cell responses, without the suppression of host immunity.

Recent efforts have concentrated on developing TNFα inhibitors that are efficient, safe and specific. The collaboration between Rockefeller University and Celgene Corporation scientists has led to the discovery of two different classes of immunomodulators derived from thalidomide and selected for their potent anti-TNFα inhibitory activity. Preliminary results indicate that at least some of these new compounds are non-toxic and non-teratogenic. The two classes of thalidomide analogues, however, possess distinct properties. IMiDs are potent inhibitors of monocyte inflammatory cytokine production and also are strong costimulators of T cell activity. SelCIDs, on the other hand, are potent PDE4 inhibitors and thus, more selective inhibitors of TNFα. Unlike IMiDs, these compounds do not costimulate T cells but inhibit T cell activity. Thus, the two classes of compounds may prove to be useful in different clinical settings according to their immunomodulatory properties. The thalidomide analogues are being used as investigational tools in animal disease models to define mechanisms of pathogenesis and to continue to elucidate the mechanisms of drug action.

We thank Dr Victoria Freedman and Dr George Muller for helpful and patient review of this manuscript, Marguerite Nulty for typing the manuscripts and Dr Patrick Haslett for critical discussions during the preparation of this report.

References:


Immunomodulation by thalidomide and thalidomide analogues

Laura G Corral and Gilla Kaplan

Ann Rheum Dis 1999 58: I107-I113
doi: 10.1136/ard.58.2008.i107

Updated information and services can be found at:
http://ard.bmj.com/content/58/suppl_1/I107

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/