

## Immunomodulation by thalidomide and thalidomide analogues

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Tumour necrosis factor  $\alpha$  (TNF $\alpha$ ), 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 TNF $\alpha$  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 TNF $\alpha$  production in vivo. For example, when leprosy patients suffering from erythema nodosum leprosum (ENL) are treated with thalidomide, the increased serum TNF $\alpha$  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 TNF $\alpha$  levels in association with an accelerated weight gain. In vitro, we have also shown that thalidomide partially inhibits TNF $\alpha$  produced by human peripheral blood mononuclear cells (PBMC) responding to stimulation with lipopolysaccharide (LPS). Recently, we found that thalidomide can also act as a costimulatory signal for T cell activation in vitro resulting in increased production of interleukin 2 (IL2) and interferon  $\gamma$  (IFN $\gamma$ ). 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 costimulatory 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-TNF $\alpha$  activity that have reduced or absent toxicities, novel TNF $\alpha$  inhibitors were designed using 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 TNF $\alpha$  inhibitors. One class of compounds, shown to be potent phosphodiesterase 4 (PDE4) inhibitors, are selective TNF $\alpha$  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 TNF $\alpha$  production, but do not inhibit PDE4 enzyme. These compounds are also potent inhibitors of several

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 TNF $\alpha$  inhibitors may potentially allow them to be used in the clinic for the treatment of a wide variety of immunopathological disorders of different aetiologies.

### TNF $\alpha$ is a key player in the immune response

TNF $\alpha$  is a pleiotropic cytokine produced primarily by monocytes and macrophages, but also by lymphocytes and NK cells. TNF $\alpha$  plays a central part in the host immune response to viral, parasitic, fungal and bacterial infections. The importance of TNF $\alpha$  and TNF $\alpha$  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 TNF $\alpha$  have a significantly reduced humoral immune response to adenovirus infection.<sup>1</sup> In *Leishmania major* infection, TNF $\alpha$  signalling is important for protection as mice lacking TNF $\alpha$  p55 receptor (TNFR-p55) show delayed elimination of the parasites compared with controls and the lesions formed failed to resolve.<sup>2</sup> Mice deficient in TNFR-p55 are also significantly impaired in their ability to clear infection with *Candida albicans* and readily succumb to the infection. TNF $\alpha$  signalling is also crucial in resisting *Streptococcus pneumoniae* infections in mice.<sup>3</sup> In addition, TNF $\alpha$  is essential for protection against murine tuberculosis. TNFR-p55 deficient mice have been shown to be more susceptible to tuberculosis infection. When TNF $\alpha$  was neutralised in vivo by monoclonal antibodies impaired protection against mycobacterial infection was observed.<sup>4,5</sup> The data from both models also established that TNF $\alpha$  and the TNFR-p55 are essential for production of reactive nitrogen intermediates by macrophages early in infection.

### TNF $\alpha$ contributes to disease pathogenesis

Although TNF $\alpha$  is crucial to the protective immune response, it also plays a part in the pathogenesis of both infectious and autoimmune diseases. Increased concentrations of TNF $\alpha$  have been shown to trigger the lethal effects of septic shock syndrome.<sup>6</sup> TNF $\alpha$  has also been implicated in the development of cachexia, the state of malnutrition that complicates the course of chronic infections and many cancers.<sup>7</sup> In rheumatoid arthritis, TNF $\alpha$  is a

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Recently, it has been shown that treatment of patients with neutralising anti-TNF $\alpha$  antibodies produces a dramatic reduction in disease activity in this condition.<sup>8</sup> Similarly, it has been shown that in inflammatory bowel disease, neutralisation of TNF $\alpha$  results in a profound amelioration of clinical symptoms.<sup>9,10</sup> Reductions in TNF $\alpha$  levels have also been linked with a significant reduction of clinical symptoms in leprosy patients with ENL, including fever, malaise, and arthritic and neuritic pain.<sup>11</sup> In tuberculosis patients, reduction of TNF $\alpha$  levels was associated with accelerated weight gain.<sup>12</sup>

#### **Thalidomide inhibits TNF $\alpha$ production by monocytes**

The pathology associated with TNF $\alpha$  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 TNF $\alpha$  production, but are also often broadly immunosuppressive (for example, cyclosporin A and corticosteroids) and therefore associated with extensive side effects.<sup>13</sup> Drugs that are potentially more specific in inhibiting TNF $\alpha$  are under active investigation and development. Our previous work has shown that the drug thalidomide ( $\alpha$ -N-phthalimidoglutarimide) is a relatively selective inhibitor of TNF $\alpha$  production by human monocytes *in vivo*. 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 TNF $\alpha$  levels. Thalidomide treatment of patients with ENL was shown to induce a prompt reduction of TNF $\alpha$  serum levels with a concomitant abrogation of clinical symptoms.<sup>11</sup> Furthermore, in patients with tuberculosis, with or without concomitant HIV infection, thalidomide treatment was found to both decrease plasma TNF $\alpha$  protein levels as well as monocyte TNF $\alpha$  mRNA levels. This decrease was associated with an accelerated weight gain.<sup>12</sup> In a rabbit model of mycobacterial meningitis, thalidomide treatment combined with antibiotics produced a marked reduction in TNF $\alpha$  levels, leucocytosis, and brain disease.<sup>14</sup> In addition, thalidomide inhibited TNF $\alpha$  serum levels in mice challenged with LPS thus partially protecting the animals from septic shock.<sup>15</sup>

*In vitro*, we have found that thalidomide selectively reduces the production of TNF $\alpha$  by human monocytes cultured in the presence of both LPS and mycobacterial products.<sup>16</sup> However, this inhibition was only partial (50% to 70%) possibly because of the instability of the drug in aqueous solutions.<sup>17</sup> The mechanism by which thalidomide reduces TNF $\alpha$  production is still unclear. The drug seems to inhibit TNF $\alpha$  production by human monocytes *in vitro* in association with enhanced degradation of TNF $\alpha$  mRNA.<sup>18</sup> It also inhibits the activation of the nuclear factor  $\kappa$ B (Nf $\kappa$ B),<sup>19,20</sup> a pro-

#### **Thalidomide has T cell costimulatory properties**

Recently, we reported that thalidomide also has a hitherto unappreciated immunomodulatory effect: the drug was shown to costimulate human T cells *in vitro*, synergising with stimulation via the T cell receptor complex to increase IL2 mediated T cell proliferation and T cell IFN $\gamma$  production.<sup>23</sup> Optimal T cell activation requires two signals.<sup>24</sup> The first signal or signal 1 is delivered by clustering of the T cell antigen-receptor-CD3 complex through engagement of specific foreign peptides bound to MHC molecules on the surface of an antigen presenting cell (APC). Signal 1 can be mimicked by crosslinking the T cell receptor (TCR) complexes with anti-CD3 antibodies. Signal 2 (or costimulation) is antigen independent and may be provided by cytokines or by surface ligands on the APC that interact with their receptors on the T cell. Costimulatory signals are essential to induce maximal T cell proliferation and secretion of cytokines, including IL2, which ultimately drive T cell clonal expansion. As antigenic stimulation in the absence of costimulatory signals leads to T cell anergy or apoptosis, costimulation is critically important in the induction and regulation of cellular immunity.

Thalidomide appears to act as a costimulator to T cells that have received signal 1 via the TCR.<sup>23</sup> In our experiments *in vitro*, stimulation of purified T cells with anti-CD3 antibodies, in the absence of signal 2, induced only minimal T cell proliferation. However, the addition of thalidomide to this cell culture system resulted in a concentration dependent increase in proliferative responses.<sup>23,25</sup> The thalidomide mediated costimulation of T cell proliferation was accompanied by increases in IL2 and IFN $\gamma$  production. It is noteworthy that in the absence of anti-CD3, there was no T cell proliferative response to thalidomide, indicating that the drug is not mitogenic in itself. It is also interesting to note that in these experiments, thalidomide did not inhibit TNF $\alpha$  production by purified T cells stimulated by anti-CD3 antibodies. This is in contrast with the effects of the drug on TNF $\alpha$  produced by monocytes. As already described above, thalidomide inhibits monocyte TNF $\alpha$  production. The costimulatory effect of thalidomide was greater on the CD8+ T cells than on the CD4+ T cell subset.<sup>23</sup>

In addition to its effects on T cell proliferation and T cell cytokine production, we observed that thalidomide induced the up-regulation of CD40L expression on activated T cells.<sup>25,26</sup> CD40L/CD40 interaction occurs early in the sequence of signalling events between T cells and antigen presenting cells (APC). Signalling through CD40 has been shown to activate APC and to induce expression of costimulatory molecules such as B7, as well as stimulating production of IL12.<sup>27,28</sup> Thus, CD40 signalling results in a stimulatory feedback mechanism in which the activated

tial for the survival of CD8+ T cells and that in its absence these cells die or become anergic.<sup>30</sup>

These studies show that in addition to its inhibitory effect on the production of monocyte cytokines, thalidomide exerts a costimulatory 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 monocyte cytokines, including TNF $\alpha$ , and the costimulation of T cell activity. The effects of thalidomide in vivo in HIV infected patients seem to reflect the costimulatory activity of the drug.<sup>26</sup> In a placebo controlled study to evaluate the effects of in vivo immunomodulation with thalidomide, the drug was administered for four weeks to HIV infected patients. Thalidomide treatment did not affect TNF $\alpha$  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 IFN $\gamma$  suggesting an immunostimulatory effect of the drug.<sup>12</sup> Recently, patients suffering from sarcoidosis have shown consistent increases in sIL2R plasma levels after thalidomide treatment (Oliver *et al*, manuscript in preparation). In the same study, thalidomide treatment increased the proliferation of sarcoid 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,<sup>23–25</sup> as well as in the T cells of HIV infected patients.<sup>26</sup>

#### Thalidomide analogues are improved TNF $\alpha$ inhibitors

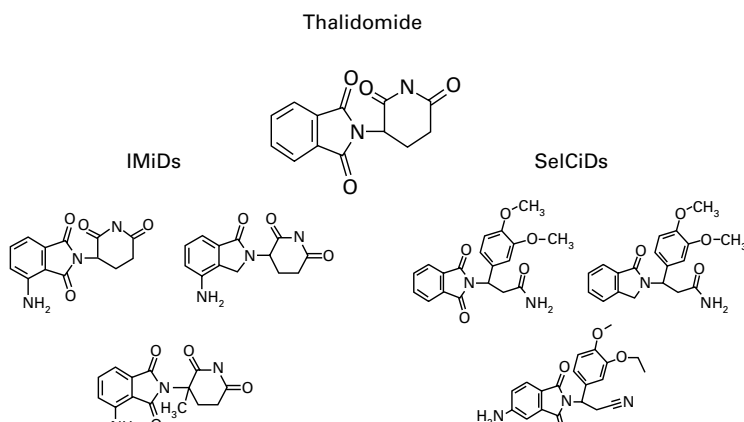
In addition to being the drug of choice for the treatment of ENL, thalidomide has been shown to be useful in a number of clinical situations including rheumatoid arthritis, HIV associated aphthous ulcers and chronic graft versus host disease.<sup>31–34</sup> However, thalidomide is a potent teratogen and ingestion of the drug by a pregnant woman can lead to catastrophic

birth defects.<sup>35</sup> In addition, thalidomide treatment is often accompanied by a number of side effects, including peripheral neuropathy.<sup>36</sup> Therefore, the use of thalidomide requires strict monitoring of all patients.<sup>37</sup> Thus, there is a pressing need to develop drugs with increased TNF $\alpha$  inhibitory activity and reduced or absent toxicities. Towards this end, structural analogues of thalidomide have been designed and synthesised at Celgene Corporation (Warren, New Jersey) and screened for inhibition of TNF $\alpha$  production. A large number of potent novel TNF $\alpha$  inhibitors were thus identified. Recently, some of these compounds were described.<sup>20–38–40</sup> 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 TNF $\alpha$  production 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.<sup>40</sup> In vivo, several of these new compounds showed improved activity in reducing LPS induced TNF $\alpha$  levels in mice<sup>17</sup> and in inhibiting the development of adjuvant arthritis in rats.<sup>40a</sup>

#### Thalidomide analogues comprise two distinct classes of molecules

A group of thalidomide analogues, selected for their capacity to potentially inhibit TNF $\alpha$  production 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.<sup>25</sup> One class of compounds, class I or ImiDs (Immunomodulatory Imide Drugs) showed not only potent inhibition of TNF $\alpha$  but also marked inhibition of LPS induced monocyte IL1 $\beta$  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 compounds, class II or SelCiDs (Selective Cytokine Inhibitory Drugs), while still potentially inhibiting TNF $\alpha$  production, had a more modest inhibitory effect on LPS induced IL1 $\beta$  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, SelCiDs were more similar to thalidomide than ImiDs.<sup>16–17</sup>

Further characterisation of the SelCiDs showed that they are potent PDE4 inhibitors.<sup>39</sup> PDE4 is one of the major phosphodiesterase isoenzymes found in human myeloid and lymphoid lineage cells.<sup>41</sup> 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 TNF $\alpha$ .<sup>42</sup> Increasing intracellular cAMP levels have been shown to inhibit TNF $\alpha$  production



regulated. Interestingly, the IMiDs and thalidomide were found not to inhibit PDE4.<sup>40</sup>

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 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.<sup>44</sup> 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.<sup>25</sup> 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 IFN $\gamma$  and induced the up-regulation of CD40L expression on T cells.<sup>25</sup> 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.<sup>25 45</sup> In vivo, however, thalidomide treatment of HIV infected<sup>26</sup> 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.<sup>28 46</sup> When T cells were stimulated by anti-CD3, thalidomide and IMiDs treatment caused a significant stimulation of IL12 production.<sup>25</sup> Thalidomide and IMiDs also induced an up-regulation of CD40L on the surface of T cells.<sup>25 26</sup> Blockade of this pathway inhibits the production of IL12 and abolishes the stimulatory effect of thalidomide.<sup>26</sup> 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.<sup>26</sup> 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.<sup>47</sup> Similarly, the paradoxical worsening of graft versus host disease<sup>48</sup> and toxic epidermal necrolysis<sup>49</sup> 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 TNF $\alpha$  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,<sup>50</sup> atopic dermatitis<sup>51</sup> and rheumatoid arthritis.<sup>52</sup> 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.<sup>40a</sup> The suppression of arthritis was accompanied by a reduction in TNF $\alpha$  and IL2 mRNA levels in the ankle joints of treated rats.

Table 1 Immunomodulatory profiles of thalidomide and thalidomide analogues

Thalidomide	IMiDs	SelCiDs
Inhibits LPS induced inflammatory cytokines TNF $\alpha$ and IL12	Strongly inhibit LPS induced inflammatory cytokines: TNF $\alpha$ , IL1 $\beta$ , IL6 and IL12	Strongly inhibit LPS induced inflammatory cytokines TNF $\alpha$ and IL12
Stimulates LPS induced anti-inflammatory cytokine IL10	Strongly stimulate LPS induced anti-inflammatory cytokine IL10	Stimulate LPS induced anti-inflammatory cytokine IL10
Costimulates T cell activation	Strongly costimulate T cell activation	Inhibit or have no effect on T cell activation

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.<sup>53–54</sup> 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.<sup>55</sup> Intensive effort is being directed towards identifying compounds with improved therapeutic 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.<sup>56–57</sup> IL12 has been shown to restore HIV specific cell mediated immunity *in vitro*<sup>58</sup> and to increase HIV specific CTL responses *in vitro*<sup>59</sup> and *in vivo*.<sup>60</sup> Also, deficient IL12 responses in HIV infected patients can be restored *in vitro* by CD40L and IFN $\gamma$ ,<sup>61</sup> 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-tumour activity in murine tumour models through various mechanisms including the stimulation of natural killer cell activity,<sup>62</sup> activation of CD8+ cytotoxic T cells<sup>63</sup> and increased IFN $\gamma$  mediated anti-angiogenesis.<sup>64</sup> Thalidomide has also recently been reported to exhibit anti-tumour activity through the inhibition of angiogenesis *in vivo*.<sup>65–68</sup> However, this anti-angiogenic effect does not seem to be mediated by TNF $\alpha$  inhibition. Although these studies did not determine the mechanism of thalidomide's anti-angiogenic activity, it is conceivable that stimulation of IFN $\gamma$ /IL12 levels may be at least partly responsible. One report indicates that thalidomide may have anti-angiogenic activity in multiple myeloma in humans.<sup>69</sup>

In summary, our recent findings that thalidomide and IMiDs preferentially costimulate CD8+ T cells and induce T cell dependent IL12 production suggest possible applications of these drugs in the control of viral infections<sup>70–71</sup> or in boosting anti-tumour immunity.<sup>72–73</sup> Also, there are anecdotal reports of the efficacy of thalidomide in treating refractory inflammatory bowel disease.<sup>74–76</sup> Recently, preliminary findings were announced from a pilot study with patients with Crohn's disease

(lando, FL). In this study, two third of the patients experienced a significant improvement in their condition. This therapeutic effect may be a combination of TNF $\alpha$  inhibition and CD8+ T cell stimulation.<sup>77–78</sup>

## Conclusions

In several disease conditions such as septic shock, chronic infections and cancer, overproduction of TNF $\alpha$  is accompanied by severe toxicities. Thalidomide inhibits TNF $\alpha$  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 TNF $\alpha$  production and/or the stimulation of T cell responses, without the suppression of host immunity.

Recent efforts have concentrated on developing TNF $\alpha$  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-TNF $\alpha$  inhibitory activity. Preliminary results indicate that at least some of these new compounds are non-toxic and non-teratogenic.<sup>20</sup> 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 TNF $\alpha$ . 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.

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- 1 Elkon K. Tumor necrosis factor alpha plays a central role in immune-mediated clearance of adenoviral vectors. *Proc Natl Acad Sci USA* 1997;94:9814–19.
- 2 Nashleas M, Kanaly S, Scott P. Control of Leishmania major infection in mice lacking TNF receptors. *J Immunol* 1998;160:5506–13.
- 3 O'Brien D. Tumor necrosis factor alpha receptor I is important for survival from Streptococcus pneumoniae infections. *Infect Immun* 1999;67:595–601.
- 4 Flynn JL, Goldstein MM, Chan J, *et al.* Tumor necrosis factor-alpha is required in the protective immune response against Mycobacterium tuberculosis in mice. *Immunity* 1995;2:561–72.
- 5 Kindler V, Sappino AP, Grau GE, Piguet PF, Vassalli P. The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. *Cell* 1989;56:731–40.
- 6 Waage A, Steinshamn S. Cytokine mediators of septic infections in the normal and granulocytopenic host. *Eur J Haematol* 1993;50:243–9.
- 7 Tracey KJ, Cerami A. Tumor necrosis factor and regulation of metabolism in infection: role of systemic versus tissue levels. *Proc Soc Exp Biol Med* 1992;200:233–9.
- 8 Maini RN, Elliott MJ, Brennan FM, Feldmann M. Beneficial effects of tumour necrosis factor-alpha (TNF-

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