

Granulocyte-macrophage colony-stimulating factor modulates immune function and improves survival after experimental thermal injury

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Thermal injury is associated with reduced colony-stimulating activity, which correlates with increased susceptibility to infection. To assess the effect of therapeutic administration of granulocyte-macrophage colony-stimulating factor (GM-CSF), 8-week old anaesthetized mice were subjected to either a 20 per cent body surface burn or a sham burn. Animals were subsequently treated with either vehicle or a range of doses of GM-CSF (10-1000 ng) with or without indomethacin (5 µg). Sepsis was induced by caecal ligation and puncture on day 10 after injury. Survival was significantly better in animals

treated with 200 ng GM-CSF on days 5-9 after the burn. Concanavalin A-stimulated T cell proliferation and interleukin (IL) 2 production were significantly depressed after burn injury. *In vivo* therapy with 200 ng GM-CSF, however, led to a significant improvement in both of these parameters of T cell function. These data suggest that GM-CSF has a potential therapeutic role in the prevention of death from burn sepsis and appears to act, at least in part, by restoring defective T cell proliferation and IL-2 production.

As a result of advances in the care of burned patients over the past two decades, deaths from hypovolaemic shock, acute renal failure and metabolic wasting have fallen dramatically. However, despite the introduction of broad-spectrum antibiotics, infection remains an important cause of morbidity and death in these patients¹. The prevalence and severity of septic episodes is largely secondary to profound alterations in defence mechanisms which characteristically follow major thermal injury. Abnormalities that predispose to the development of infection include loss of the normal dermal barrier, impaired cell-mediated immunity and functional defects in mononuclear phagocytes and polymorphonuclear leucocytes²⁻⁴. Thermal injury is also associated with prolonged impairment of T cell proliferative responses and interleukin (IL) 2 synthesis, changes that correlate closely with increased susceptibility to infection⁵⁻⁸.

Although the precise mechanisms underlying these changes have not been fully elucidated, recent studies⁹⁻¹⁴ suggest that reduction in the activity of granulocyte-macrophage colony-stimulating factor (GM-CSF) plays a pivotal role in mediating many of the characteristic alterations in immune function after thermal injury. The present study explored the potential value of this cytokine when used alone and in combination with the cyclooxygenase inhibitor, indomethacin, as a therapeutic regimen designed to restore immune function and improve survival in an experimental model of thermal injury.

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Materials and methods

Burn model

Male A/J mice aged 7 weeks were acclimatized for 1 week under controlled conditions with water and mouse food *ad libitum*. Care of animals and all procedures were carried out in accordance with National Institutes of Health guidelines and with the approval of the Harvard Medical School Standing Committee on Animal Research.

After randomization animals were anaesthetized with intraperitoneal pentobarbital 0.02 mg/g. Animals were shaved on the dorsum and placed in a specially constructed mould which exposed 20 per cent of the body surface area. The mould containing the anaesthetized animal was immersed in water at either room temperature (25°C) (sham) or 90°C (burn) for 9 s according to the treatment protocol assigned to each animal. The latter treatment resulted in a histologically proven full-thickness insensate burn to the exposed dorsum. Animals were immediately dried and resuscitated with 1 ml 0.9 per cent saline intraperitoneally.

Survival studies

Septic challenge was induced on day 10 after the burn, which corresponds with the point of maximum impairment in T cell proliferative responses and maximum susceptibility to infectious challenge in this model⁵. Animals were anaesthetized and the abdomen was shaved and opened in the midline. The caecum was located and ligated at its base in a non-obstructing fashion using a 4/0 silk suture. A full-thickness puncture, at two different sites in the ligated caecum, was performed with a 27-G needle. A small quantity of faeces was expressed from each puncture wound to ensure peritonitis.

After caecal ligation and puncture (CLP) the caecum was replaced in the abdomen, taking care not to contaminate the wound with faeces. The wound was closed with a continuous 5/0 nylon suture. All animals were immediately resuscitated with 1 ml 0.9 per cent saline intraperitoneally. The animals were observed for at least 14 days after operation and survival curves calculated.

Experimental groups

The following three groups were studied: (1) sham burn animals treated with vehicle (0.9 per cent saline); (2) animals with burn injury treated with vehicle; and (3) those with burn injury treated with GM-CSF (10, 50, 200, 500 and 1000 ng) with or without 5 µg indomethacin.

Vehicle (0.9 per cent saline) and therapy were administered on days 0–5 or 5–9, by either intraperitoneal or subcutaneous injection, in a final volume of 0.5 ml (12–15 animals per group). Highly purified, endotoxin-free GM-CSF was obtained as a gift (Amgen, Thousand Oaks, California, USA). Indomethacin was prepared in 0.9 per cent saline.

Splenocyte preparation

For *in vitro* studies of T cell function, animals were killed in a carbon dioxide chamber on day 10 after injury. The spleen was removed and a splenocyte suspension obtained by teasing apart the splenic matrix. Cells were suspended in RPMI 1640 culture medium containing 2-mercaptoethanol 5×10^{-3} mol/l, l-glutamine 2 mmol/l, HEPES buffer 10 mmol/l and antibiotic-antimycotic solution 10 ml/l containing penicillin 10000 units, streptomycin 10000 µg/ml and amphotericin B 25 µg/ml. The cells were washed three times (10 min, 400g, 4°C). Supernatants were discarded and the cell pellets from individual animals were resuspended in 2 ml complete medium (wash medium and 5 per cent fetal calf serum heat inactivated by incubating at 56°C for 30 min and filtered using a 0.45-µm filter). Mononuclear cells were counted and plated in 96-well microtitre plates (100 µl per well) at a final concentration of 2×10^5 cells per well. Cell viability was routinely estimated as greater than 95 per cent using the trypan blue dye exclusion test.

T cell proliferation and interleukin 2 production

To each well 100 µl complete medium containing concanavalin A 5 µg/ml was added. Plates were incubated at 37°C in 5 per cent carbon dioxide.

When assaying for IL-2, 150 µl supernatant from each well was removed after 24 h. Supernatants from quadruplicate wells were pooled and frozen at -70°C until assay. IL-2 was measured using the technique described by Gills *et al.*¹⁵. A 100 µl volume of sample was added to 96-well tissue culture plates. Samples were serially diluted with complete medium (1:2 to 1:64 dilution), and 100 µl cells (5×10^5 per ml) from the cytotoxic T lymphocyte line (CTLL) was added to each well.

Cultures containing both (CTLL) cells and the diluted samples were incubated at 37°C in 5 per cent carbon dioxide for 44 h. A 25 µl volume of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (5 mg/ml in phosphate-buffered saline) was added to each well. Plates were cultured for a further 4 h. Supernatant (100 µl) from each well was then removed, and 150 µl acidified 10 per cent sodium dodecyl sulphate solution (pH 4.5) was added to lyse the cells and solubilize the formazan dye that is produced from MTT by proliferating cells. The optical density (at 650–570 nm) of each well was read on a fully automated 96-well microenzyme-linked immunosorbent assay plate reader (Dynatech microplate reader; Molecular Devices, Menlo Park, California, USA). Units of IL-2 were determined by comparison with a standard curve produced by a standard solution of T cell growth factor. This was assigned an arbitrary value of 1 unit. The log₂ of the dilution producing 50 per cent of the maximum reading for the standard solution was determined by probit analysis.

Proliferation was measured by both calculating the cellular incorporation of tritiated thymidine and measuring reduction of MTT as described above. After culture for 30 h at 37°C in 5 per cent carbon dioxide a pulse of 1 µCi [³H]thymidine in 25 µl complete medium was added to each well. Plates were cultured for a further 18 h before rapid freezing to -20°C. Plates were subsequently thawed and cells harvested using a multiautomated cell harvester (Cambridge Technology, Cambridge, Massachusetts, USA). Incorporation of [³H]thymidine was calculated by measuring the activity in a liquid scintillation counter.

Statistical analysis

Survival following CLP was plotted using Kaplan–Meier curves. A log rank test was initially performed to compare the survival curves of all groups within each experiment before calculating the Wilcoxon–Gehan survival statistic to compare the survival curves of individual groups.

Analysis of variance was performed in experiments with multiple groups before comparisons were made between groups using the Sidak adjustment for multiple *t* tests. Statistical analysis was carried out with the Stata statistics program (Computing Resource Centre, Santa Monica, California, USA).

Results

Survival studies

The mortality rate after burn injury alone was 20 per cent compared with 0 per cent after sham burn. Caecal ligation and puncture in sham-burned animals consistently yielded a mortality rate of 20–40 per cent (Figs 1–3). In contrast, animals subjected to CLP on day 10 after the burn all died ($P < 0.01$, Wilcoxon–Gehan survival test). Post-mortem examination of non-survivors revealed generalized peritonitis, whereas survivors, killed for examination after 14 days, displayed localized inflammatory masses in the region of the caecum.

Initial studies examined the effect of subcutaneous therapy administered on days 5–9 after the burn (Fig. 1). Low-dose GM-CSF (10 and 50 ng) conferred no significant beneficial effect on survival. High-dose therapy (500 and 1000 ng) had no detectable effect on survival after thermal injury. However, burned animals treated with 200 ng GM-CSF displayed a significant improvement in survival rate compared with those treated with vehicle only (0 versus 33 per cent respectively, $P < 0.05$).

The addition of indomethacin to the therapeutic regimen did not further enhance survival in groups treated with 200 ng GM-CSF (Fig. 2). The use of indomethacin alone had no discernible effect on survival.

Route of administration. To assess the importance of the route of administration, survival was determined after intraperitoneal and subcutaneous therapy (Fig. 3). GM-CSF 200 ng/day was used as this dose was most effective

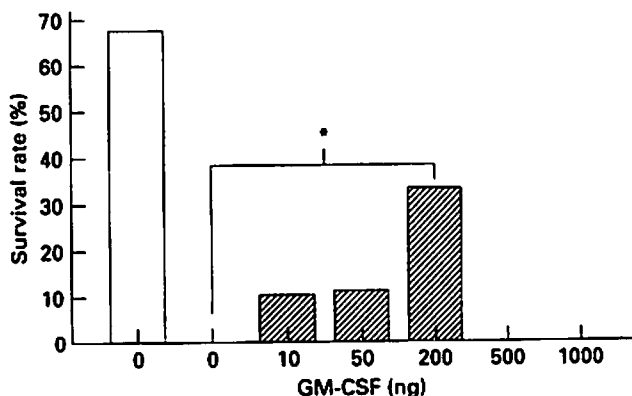


Fig. 1 Survival in sham (□, *n* = 12) and burn (■, *n* = 14) groups following caecal ligation and puncture on day 10 after burn injury. Treatment groups included sham-burned animals treated with vehicle (0.9 per cent saline) and burned animals receiving either vehicle or increasing doses of granulocyte-macrophage colony-stimulating factor (GM-CSF); therapy was administered on days 5–9 after injury by subcutaneous injection. * $P < 0.05$ (Wilcoxon–Gehan survival statistic)

when used alone or in combination with indomethacin. Burned animals appeared to display a greater improvement in survival rate when GM-CSF was administered by intraperitoneal injection compared with subcutaneous treatment (47 versus 29 per cent respectively).

Timing and duration of therapy. Burned animals were treated with 200 ng GM-CSF, via both intraperitoneal and subcutaneous routes, on days 0–5 after injury before CLP was performed on day 10. There was no significant improvement in survival rate in GM-CSF treatment groups using this regimen.

Weight loss after thermal injury

The mean weight of sham-burned animals was unaltered after injury (Fig. 4). Mice receiving a burn, however,

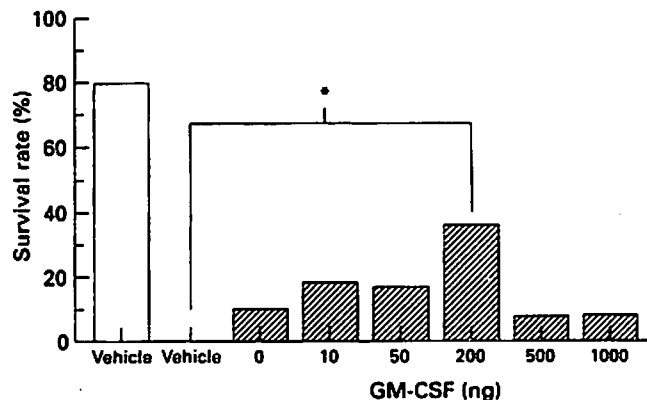


Fig. 2 Survival in sham ($n = 10$) and burn ($n = 14$) groups following caecal ligation and puncture on day 10 after burn injury. Treatment groups included sham-burned animals treated with vehicle (0.9 per cent saline) (□) and burned animals receiving either vehicle or 5 µg indomethacin, and increasing doses of granulocyte-macrophage colony-stimulating factor (GM-CSF) (▨); therapy was administered on days 5–9 after injury by subcutaneous injection. * $P < 0.05$ (Wilcoxon–Gehan survival statistic)

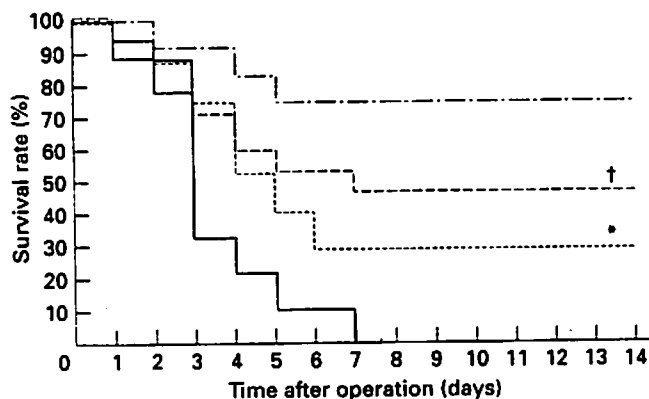


Fig. 3 Survival in sham ($n = 12$) and burn ($n = 14$) groups following caecal ligation and puncture on day 10 after burn injury. Treatment groups included sham-burned animals treated with vehicle (— · — · —) and burned animals receiving vehicle (—) or 200 mg granulocyte-macrophage colony-stimulating factor (GM-CSF) by either intraperitoneal (— — —) or subcutaneous (· · · · ·) routes; therapy was administered on days 5–9 after injury. * $P < 0.05$, † $P < 0.01$ versus burn group treated with vehicle (Wilcoxon–Gehan survival statistic)

displayed a significant fall in weight, which amounted to a 9 per cent reduction in total body-weight 10 days after thermal injury. GM-CSF and indomethacin therapy appeared to protect against weight loss, an action that was most marked in groups treated with 200 ng GM-CSF and 5 µg indomethacin. GM-CSF therapy in the absence of indomethacin had a similar effect on weight loss (data not shown). The weight loss in burn groups treated with either vehicle or GM-CSF with indomethacin appeared to be inversely related to survival after CLP.

T cell proliferative responses after thermal injury

Concanavalin A-stimulated T cell proliferation (measured by MTT assay and incorporation of tritiated thymidine) was significantly lower in burn groups than in sham-burned controls (Figs 5 and 6).

In vivo administration of granulocyte-macrophage colony-stimulating factor with and without indomethacin. In vivo treatment of burned animals with 200 ng GM-CSF on days 5–9 significantly restored T cell proliferative responses (Fig. 5). Single-agent therapy with either indomethacin or GM-CSF had a beneficial effect on T cell proliferation, but combined therapy with the two agents did not further enhance proliferative responses. GM-CSF therapy had no significant action on T cell proliferative responses when administered on days 0–5 after injury (data not shown).

In vitro culture with granulocyte-macrophage colony-stimulating factor. Splenocytes from both sham and burn groups were also cultured in the presence of increasing concentrations of GM-CSF *in vitro*. The addition of GM-CSF to cultures consistently enhanced T cell proliferation, using both the MTT (Fig. 6) and tritiated thymidine assays.

Interleukin 2 synthesis

Thermal injury was associated with a significant reduction in IL-2 production (Figs 7 and 8). Splenocytes from thermally injured animals treated *in vivo* with GM-CSF

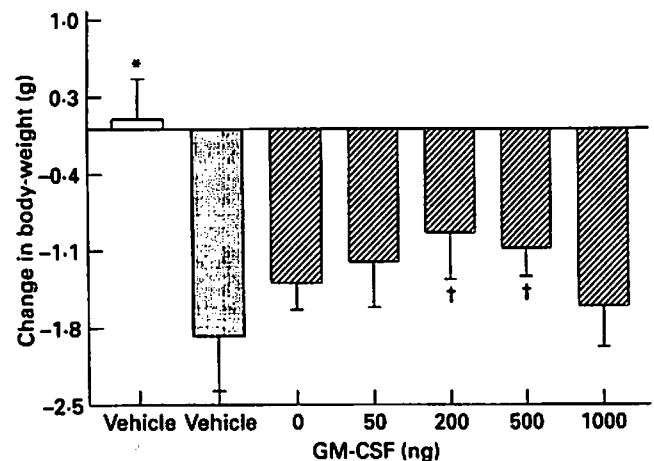


Fig. 4 Mean (s.e.m.) change in weight on day 10 after burn injury in sham (□, $n = 10$) and burn ($n = 14$) groups treated *in vivo* with vehicle (□) or granulocyte-macrophage colony-stimulating factor (GM-CSF) and 5 µg indomethacin (▨). Data from same experiment as Fig. 2. * $P < 0.001$ versus all other groups; † $P < 0.05$ versus burn group treated with vehicle (t test)

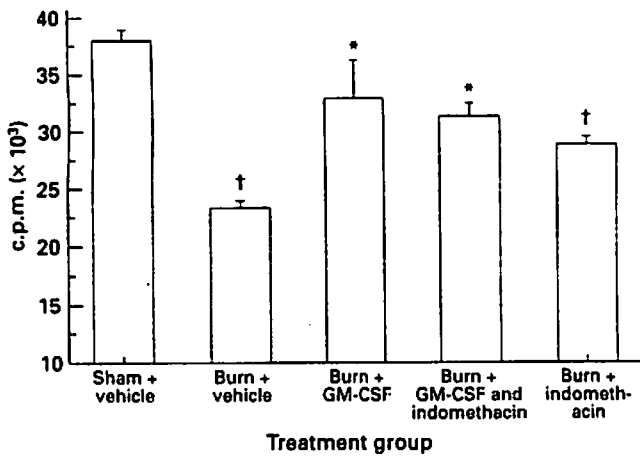


Fig. 5 Concanavalin A (2.5 µg/ml)-stimulated T cell proliferation measured using incorporation of tritiated thymidine (n = 12–14 per group). Treatment groups include sham-burned animals treated with vehicle (0.9 per cent saline) and burn groups receiving either vehicle, 200 ng granulocyte-macrophage colony-stimulating factor (GM-CSF), 200 ng GM-CSF plus 5 µg indomethacin, or 5 µg indomethacin. All therapy was administered by subcutaneous injection on days 5–9 after the burn. The spleen was harvested on day 10 after injury. Proliferation for each animal was measured in quadruplicate before calculation of the group mean (s.e.m.). *P < 0.01 versus burn group treated with vehicle; †P < 0.05 versus sham group receiving vehicle (t test)

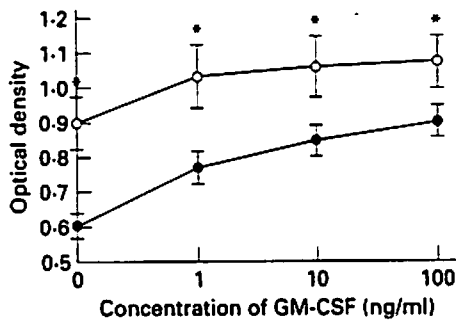


Fig. 6 Concanavalin A (2.5 µg/ml)-stimulated T cell proliferation in cultures from sham (○) and burn (●) groups in the presence of *in vitro* granulocyte-macrophage colony-stimulating factor (GM-CSF) using the MTT assay. Spleens were harvested on day 10 after the burn injury. Results represent the mean (s.e.m.) of four determinations for each concentration of GM-CSF. Cells from sham and burn groups (n = 5 per group) were pooled before assay. *P < 0.01 (sham versus burn group, t test)

(200 ng) displayed a significant increase in IL-2 production (Fig. 7). *In vivo* treatment of burned animals with the combination of indomethacin and GM-CSF (200 and 500 ng) on days 5–9 also upregulated IL-2 synthesis (Fig. 8). Combination therapy with indomethacin and high-dose (1000 ng/day) or low-dose (50 ng/day) GM-CSF did not, however, alter synthesis of this lymphokine. Similarly, indomethacin therapy in the absence of GM-CSF had no apparent effect on IL-2 production (Fig. 8).

Discussion

Over the past 10 years a group of cytokines has been described that stimulates proliferation and differentiation in myelomonocytic cells. Of these colony-stimulating

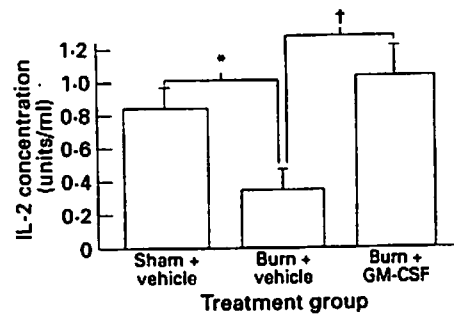


Fig. 7 Interleukin (IL) 2 production in sham-burned animals treated with vehicle (0.9 per cent saline) and burned animals receiving either vehicle or 200 ng granulocyte-macrophage colony-stimulating factor (GM-CSF); therapy was administered by subcutaneous injection on days 5–9 after injury. Splenocytes were harvested on day 10 after injury. *In vitro* T cell IL-2 production was determined after stimulation for 24 h with concanavalin A 2.5 µg/ml. Values represent the mean (s.e.m.) concentration of IL-2 in supernatants of individual animals in each group (n = 12–14 per group). *P < 0.01, †P < 0.001 (t test)

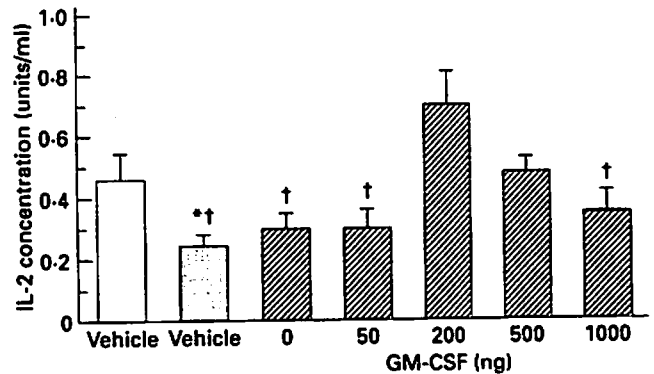


Fig. 8 Interleukin (IL) 2 production in sham-burned animals treated with vehicle (0.9 per cent saline) (□) and burned animals receiving either vehicle (□) or 5 µg indomethacin plus a range of doses of granulocyte-macrophage colony-stimulating factor (GM-CSF) (▨); therapy was administered by subcutaneous injection on days 5–9 after injury. Splenocytes were harvested on day 10 after injury. *In vitro* T cell IL-2 production was determined after stimulation of splenocytes for 24 h with concanavalin A 2.5 µg/ml. Values represent the mean (s.e.m.) concentration of IL-2 in supernatants from individual animals in treatment groups (n = 12–14 per group). *P < 0.05 versus sham-burned control group; †P < 0.01 versus burn group treated with 200 ng GM-CSF and indomethacin (t test)

factors GM-CSF also appears to play an important role in modulating immune function. More recently, a number of studies have provided direct and indirect evidence that GM-CSF activity is reduced after thermal injury, a defect that may be responsible for many of the characteristic defects in macrophage and T cell function^{9–14}.

Peterson *et al.*^{9,10} reported low serum colony-stimulating activity in association with monocytopenia in patients who developed fatal sepsis after major burn injury. Survivors displayed a prompt rise in serum levels of colony-stimulating factors which was associated with the development of monocytosis. A further report from the same group¹¹ noted an increase in the number of peripheral blood granulocytic stem cells in patients who survived burn injury. Although the levels of circulating

enhanced when administered directly at the site of subsequent septic challenge (Fig. 3). In selected experiments indomethacin was also added to the therapeutic regimen in an effort to reduce GM-CSF toxicity and improve survival. Prostaglandin synthase inhibitors such as indomethacin have been shown¹⁻²⁵ to reduce significantly the toxicity and improve the efficacy of therapy with several other cytokines including IL-1, TNF and IL-2. Indomethacin acts by downregulating synthesis of the immunosuppressive prostaglandin (PG) E₂, which inhibits colony-stimulating activity and IL-2 synthesis^{23,24,27}. Since overproduction of PGE₂ is a characteristic feature of thermal injury, and its synthesis may be further stimulated by GM-CSF, concomitant administration of a prostaglandin synthase inhibitor such as indomethacin might have enhanced the efficacy of therapy. Surprisingly, combination therapy did not improve survival compared with treatment with GM-CSF alone (Figs 1 and 2). In keeping with the findings of others^{24,25}, indomethacin therapy on its own had no significant effect on survival.

Like others^{5,8,19,25,27}, the present study documented a significant reduction in T cell proliferative responses and IL-2 production after thermal injury (Figs 5-8). GM-CSF therapy *in vivo* led to a significant improvement in T cell proliferation (Fig. 5). In addition, concanavalin A-stimulated splenocyte cultures from sham and burn groups displayed a classical dose-response curve to GM-CSF *in vitro*, which strongly suggests a direct action on T cell function. Although T cell proliferation is essential for the development of an adequate response to infection, IL-2 plays a pivotal role in maintaining and amplifying this response²⁸. Impaired production of this cytokine has been associated with immunosuppression in patients with multiple trauma, thermal injury, cancer, acquired immune deficiency syndrome and old age^{5,23,27,29-31}. In the present study *in vivo* GM-CSF therapy, both alone and in combination with indomethacin, restored defective IL-2 synthesis after thermal injury (Figs 7 and 8). It is also interesting to note that the dose of GM-CSF that conferred the best survival advantage after CLP also produced optimal restoration in both T cell proliferation and IL-2 production.

Although the combination of either IL-1 or IL-2 with a cyclo-oxygenase inhibitor has been shown to enhance survival after experimental burn sepsis, an interval of at least 5 days is required between administration of the last treatment and induction of the septic challenge^{24,25}. The requirement for a latent period implies a secondary action. Recent data suggest that both of these therapies may act by stimulating the release of GM-CSF^{17,23,32}. In the present study there was no such requirement for a latent period between cessation of therapy and therapeutic effect. In fact, therapy was ineffective when stopped 5 days before the septic challenge. The present results suggest that *in vivo* GM-CSF therapy on days 5-9 after the burn injury mediated its beneficial effects on survival by restoring defective T cell proliferative responses and IL-2 production. The failure of therapy on days 0-5 either to improve survival or to restore defective T cell function implies a direct but transient action on T lymphocytes. This is confirmed, in part, by the ability of GM-CSF to enhance T cell proliferation when added directly to cultures (Fig. 6).

Although T lymphocytes are not conventionally considered to be one of the primary targets of GM-CSF, a number of recent studies have suggested that this

colony-forming units are universally depressed during the initial 48 h after injury, they return to normal or supra-normal values in patients with non-fatal burns. However, non-survivors display a persistent reduction in the level of colony-forming units, which is further depressed in non-survivors who develop septicæmia or burn wound sepsis due to Gram-negative bacteria and/or fungi¹¹. These data provide evidence of defective stem cell production and/or differentiation in patients with severe thermal injury.

In clinical practice the vast majority of septic complications occur in the second or subsequent weeks after thermal injury. Because there is no accurate method of predicting whether or when injured patients might develop a septic complication, GM-CSF therapy was administered up to the point of septic challenge on day 10 after burn injury, which corresponds with the point of maximum immunosuppression in this model⁵. Under these conditions once-daily subcutaneous treatment with 200 ng GM-CSF on days 5-9 significantly increased the survival rate in burned animals (Fig. 1). As expected from similar studies using other cytokines, GM-CSF did not display a classical dose-response curve with respect to its *in vivo* actions. Both very high and low doses were ineffective. It is of interest to note that GM-CSF-mediated improvement in survival appears to be closely related to a concomitant restoration in T cell proliferative responses and IL-2 synthesis. In addition, the dose that had the greatest impact on survival also appeared to ameliorate the characteristic wasting that follows thermal injury. Although it is unclear from the present data how GM-CSF mediated these changes in nutritional status, it is worthy of note that *in vivo* therapy with tumour necrosis factor (TNF) has also been shown¹⁶ to have a similar action in a rodent model of burn sepsis. In that study animals displayed a 9 per cent reduction in weight over a 6-day period after thermal injury and pair-fed sham-burn animals displayed a similar reduction. Animals subjected to a combined burn and inoculation with *Pseudomonas aeruginosa*, however, suffered a 22 per cent reduction in mean body-weight. Surprisingly, the administration of TNF to the latter group led to a significant reduction in weight loss. It is interesting to note that TNF is a potent stimulator of GM-CSF release¹⁷.

It is unclear from the present study why higher dose regimens were less effective, but a similar phenomenon has been reported by other investigators using both GM-CSF and IL-2 therapy. French *et al.*¹⁸ noted that GM-CSF at a dose of 30 ng was more effective than either higher doses of 300 ng and 3000 ng or a reduced dose of 0.03 ng in preventing neonatal death from *Staphylococcus aureus* infection. Gough and colleagues¹⁹ noted that *in vivo* IL-2 therapy at a dose of 64 000 units per day restored T cell proliferative responses and IL-2 production in a murine model of thermal injury, whereas both higher and lower dose regimens were less effective.

Intervention using immunomodulating agents must also take into account the local effect of therapy. Intra-peritoneal administration of many agents, including IL-1, TNF- α and polymyxin, induces profound changes in local cell populations, by stimulation of the influx of granulocytes and macrophages into the peritoneal cavity. Similarly, intraperitoneal treatment with GM-CSF not only increases the concentration of macrophages and neutrophils, but also activates these cells²⁰, changes that are likely to influence the clinical course of a septic challenge within the peritoneal cavity itself. The present study has demonstrated that the efficacy of GM-CSF is

cytokine may alter T cell function by a direct action as well as modulating accessory cell function. GM-CSF can initiate and maintain long-term growth of the HT-2 cell line, which was thought to be dependent solely on IL-2 and IL-4^{33,34}. Santoli *et al.*³⁵ also noted that GM-CSF was capable of amplifying IL-2-driven T cell proliferation in both unstimulated and mitogen-activated human lymphocytes. The ability to induce growth in a T cell line and to serve as a cofactor for *in vivo* derived T cells suggests a possible physiological role for GM-CSF in T cell-mediated immune responses.

The present study shows that GM-CSF therapy confers a significant survival advantage after infectious challenge in thermally injured animals. The cytokine is effective when administered up to the point of septic challenge and appears to mediate its actions, at least in part, through a restoration in T cell proliferation and IL-2 production. These data suggest a potential therapeutic role for GM-CSF in the prevention of death after major thermal injury, although they caution against the inappropriate use of high-dose therapy.

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Randomized controlled trials

Prospective authors are requested to consult, digest and apply the Instructions to Authors in any submission they may wish to make to the Journal. The presentation of manuscripts following these guidelines greatly facilitates the editorial process. Readers and prospective authors are particularly asked to note the instructions pertaining to the identification of randomized controlled trials. The UK Cochrane Centre, along with many other institutes and journals, is keen to identify all randomized controlled trials. Current literature searches identify with certainty only 50-60 per cent of such publications from the title or abstract as published. Authors are encouraged to ensure that randomized controlled trials are fully identified as such in title and summary as requested in the Instructions to Authors. Cooperation in these matters will be appreciated by editors and referees alike.

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