Inhibition of the Immunosuppressive Activity of Resident Pulmonary Alveolar Macrophages by Granulocyte/Macrophage Colony-stimulating Factor

By N. Bilyk and P. G. Holt

From the Division of Cell Biology, Western Australian Research Institute for Child Health, Subiaco, Western Australia 6008, Australia

Summary

Resident pulmonary alveolar macrophages (PAM) play an important role in the maintenance of immunological homeostasis in the lung via downmodulation of local T cell responses in the steady state. The present study demonstrates that this pathway for T cell suppression is reversible via granulocyte/macrophage colony-stimulating factor (GM-CSF). Thus, freshly isolated PAM strongly inhibit mitogen-induced T cell proliferation, and pretreatment of the PAM with cytokine-rich lung-conditioned medium (LCM) generated by exposure of lung to bacterial lipopolysaccharide (LPS) abrogated this suppressive activity. LCM from lungs of normal and athymic nude mice exhibited identical activity. Moreover, the PAM-modulating activity of LCM was inhibited by blocking antibody specific for GM-CSF, and the activity of LCM could be reproduced by recombinant GM-CSF. This suggests that secretion of GM-CSF by mesenchymal cells and/or macrophages under stimulation from agents such as LPS provides a potential mechanism for upregulation of local T cell responsiveness during acute inflammation. In addition, experiments with a range of cytokines indicated that interleukin 4, transforming growth factor β1 and tumor necrosis factor α (TNF-α) exhibited weaker (but significant) modulatory effects on PAM, and (in the case of TNF-α) amplified the effects of GM-CSF.
effects of exogenous TNF-α, TGF-β1, and IL-4 on PAM function, which in the case of TNF-α, is additive with GM-CSF.

Materials and Methods

Animals. Female, specified pathogen-free, C3H/HeJ mice were used at 5-12 wk of age (Animal Resource Centre, Murdoch University, Western Australia). Age- and sex-matched BALB/c and C57BL/6J mice were also used where indicated.

Reagents and Cytokines. LPS (type B Escherichia coli 026:B6) was obtained from Difco Laboratories (Detroit, MI). Con A was purchased from Pharmacia (Uppsala, Sweden), and PHA was from Wellcome (Dartford, England). Recombinant murine GM-CSF was obtained from Biosource International (Camarillo, CA). Purified natural human TGF-β1 and human rTNF-α was obtained from Genzyme Corp. (Cambridge, MA). Recombinant murine TNF-α was purchased from Boehringer Mannheim GmbH (Mannheim, Germany). Murine rIL-4 was a generous gift from Dr. P. Hodgkin (John Curtin School of Medical Research, Australian National University, Canberra).

LPS-induced LCM. LCM was generated as previously described (20). Briefly, BALB/c mice were given 5 μg LPS in saline intravenously and the lungs were removed in toto 3 h later. After washing in PBS, each lung was placed in 5 ml RPMI and incubated for 48 h at 37°C. Conditioned media was pooled, heat inactivated at 56°C, clarified by centrifugation, and sterilized by passage through a 0.22-μm filter. FCS was added at a final concentration of 5%, and aliquots were stored at -20°C.

Neutralizing Antibody to GM-CSF. Polyclonal rabbit anti-murine GM-CSF was a generous gift from Dr. K. Kumagai (Department of Microbiology, School of Dentistry, Tohoku University, Sendai, Japan). This antibody has been shown previously not to crossreact with other murine CSFs (21). In preliminary experiments, we ascertained that a 1:20 dilution of antibody could inhibit the proliferation of the GM-CSF/IL-3-dependent cell line FDCP-1 induced with recombinant murine GM-CSF, and this antibody concentration was used to neutralize LCM activity. The antibody was preincubated with LCM for 1 h at 37°C, before addition to PAM. Control antibody was a polyclonal antiserum against M-CSF (generously provided by Dr. E. R. Stanley, Albert Einstein College of Medicine, Bronx, NY).

Cell Preparation. PAM were obtained as previously described (22). Briefly, lungs were inflated with warm PBS containing 0.2% BSA (Commonwealth Serum Laboratories, Melbourne, Australia) and 12 mM lignocaine (Orion Laboratories, Welshpool, Western Australia) via cannulation of the trachea, and were lavaged at least 15 times. Cells were collected by centrifugation and resuspended in RPMI 1640 (Gibco, Melbourne, Australia) containing 5% FCS, hereafter referred to as medium. Lymph node cells (LNC) were obtained from a pool of mesenteric, inguinal, and brachial lymph nodes and were processed in PBS supplemented with 0.2% BSA and resuspended in culture.

Suppression of Mitogen-induced LNC Proliferation. PAM at various concentrations were incubated in triplicate in 200 μl medium in 96-well flat-bottomed trays, with medium, LCM at 10% (vol/vol), or cytokines at the indicated concentrations, for 48 h (overall survival in the PAM cultures over this period was >95%). Medium was removed, the cells washed three times in situ, and 4 × 10⁶ LNC added to each well in 200 μl medium, containing Con A at 2.5 μg/ml; background control wells contained no Con A. Preliminary experiments demonstrated that the proliferation of LNC was unaltered in the presence of up to 50% of the various macrophage-conditioned media, confirming that there was no detectable carry-over of cytokine before the addition of LNC. Plates were incubated for 48 h, and [3H]thymidine (Amersham, Sydney, Australia) was added 16 h before harvesting the cells for determination of [3H]DNA synthesis (measured as cpm/culture).

Results

Preexposure to Lung-conditioned Medium Abrogates the Capacity of PAM to Inhibit T Cell Proliferation. Data presented below are typical of a large series of experiments. freshly prepared resident PAM are able to completely suppress the Con A-induced proliferation of LNC when introduced into the cultures at a final density equivalent to >95% of the total cells per well (Fig. 1 A). The same effect was observed using either PHA or Con A over a concentration range of 0.5-5.0 μg/ml, and is also readily demonstrable with antigen-induced T cell proliferation (data not shown). To ascertain whether lung-derived inflammatory cytokines could modulate the suppressive capacity of PAM, we generated conditioned medium from LPS-stimulated whole lung (LCM) and preincubated PAM in the wells with LCM-supplemented medium for 24 or 48 h before the addition of mitogen and LNC. Fig. 1 B shows that while PAM incubated for 48 h in medium alone maintain their capacity to suppress the mitogen-induced proliferation of LNC, PAM pretreated with LCM lose their ability to inhibit proliferation. Similar results were also obtained in BALB/c and C57BL/6J mice, and only minor differences were observed when comparing 24- and 48-h LCM treatment (data not shown). The fact that LCM from nude and normal animals were equally potent in downmodulating the suppressive activity of PAM implies that lung T cells are not an important source of the agent(s) that mediate these effects.

LCM Modulation of PAM Immunosuppressive Activity Is Abrogated by a Polyclonal Anti-GM-CSF Antibody and Is Reproduced by Recombinant GM-CSF. LCM is rich in GM-CSF (20), and so we examined whether GM-CSF itself was responsible for the modulating activity in LCM. Fig. 2 A shows that a polyclonal anti-GM-CSF was able to neutralize the majority of the PAM-modulating activity of LCM. The activity of the anti-GM-CSF antibody was inhibited by the inclusion of up to 100 ng/ml recombinant GM-CSF and the control sera directed against M-CSF had no effect on LCM activity (data not shown). In addition, we preincubated PAM with GM-CSF at various concentrations for the same time as LCM, and found that suppression was reversible by GM-CSF in a dose-dependent fashion (Fig. 2 B). Maximal effects were observed at 50 ng/ml (an average of 75% inhibition of immunosuppressive activity over 12 experiments).

GM-CSF Downmodulation of PAM-suppressive Activity Is Enhanced by TNF-α. The experiment detailed in Fig. 3 compares the immunomodulatory effects of GM-CSF with TNF-α, TGF-β1, and IL-4. The latter cytokines were used over a 10-20-fold dose range (highest concentration only shown). All three cytokines were capable of partially abrogating the suppressive effects of PAM, the most active being TNF-α, which was in the order of one-third as effective as GM-CSF.
Figure 1. LCM inhibits the lymphocytostatic activity of PAM. (A) Fresh PAM were titrated into $4 \times 10^9$ LNC containing Con A (2.5 μg/ml) and the cultures were incubated for 48 h before harvesting to determine $[^{3}H]$thymidine incorporation. (B) PAM were preincubated for 48 h in medium (●), or LCM prepared from normal (●) or athymic nude (○) mice, before the addition of LNC and Con A. Results are expressed as the mean dpm ± SD, minus background, of nine individual experiments.

A range of concentrations of IL-1 and IFN-β were also tested, but proved ineffective (not shown).

GM-CSF alone was capable of abrogating up to 75% of the immunosuppressive activity of PAM, but the combination of GM-CSF and TNF-α completely inhibited the capacity of the PAM to suppress T cell proliferation. Comparable additive effects have not been observed using TGF-β, and IL-4 (data not shown).

Discussion

This is the first report to demonstrate that a specific cytokine, viz. GM-CSF, is able to abrogate the immunosuppressive activity of resident PAM. Given that we have used a non-specific inflammatory stimulant (LPS) to induce LCM and that both normal and athymic nude animals produce LCM with similar modulating activity, it appears likely that the GM-CSF is of mesenchymal or macrophagic origin, and as such is produced at an early stage of the host response after the initial inflammatory stimulus is encountered. GM-CSF has been
shown to be secreted by both airway epithelial cells (23) and macrophages (24) via the direct stimulatory effects of LPS, and in addition, endothelial cells (25), fibroblasts (26), and airway epithelial cells (27) respond to cytokines released by LPS-stimulated macrophages (notably IL-1 and/or TNF-α) via GM-CSF secretion. All of these cell types are abundant in lung tissue, and thus potentially contribute to local immunoregulation through this pathway.

While GM-CSF appears to be the most effective cytokine with respect to modulation of the immunoregulatory functions of PAM, it is clearly not unique in this regard, as TNF-α, TGF-β1, and IL-4 all express low (but significant) modulatory activity, and (at least in the case of TNF-α) can amplify the effects of GM-CSF. Both TNF-α (28) and TGF-β1 (29) have been shown to be released in high levels by activated macrophages at sites of inflammation in the lung, and may thus contribute in an autocrine fashion to the regulation of macrophage-mediated T cell activation in these areas.

In addition, despite the apparent lack of a contribution from T cells to the PAM modulatory activity of LCM in the present experiments, earlier studies (30) have noted down-modulation of PAM suppression by lymphokine-rich T cell supernatants, but identification of the active agent(s) was not attempted. It appears likely from our current results that T cell-derived GM-CSF, IL-4, and TNF-α may account for this activity.

In relation to the mechanism of GM-CSF modulation of PAM activity, we have recently reported that one pathway for PAM-mediated suppression of T cell activation involves down-modulation of the function of the major APC population present in lung tissue (31). Future studies are required to determine whether the effects of GM-CSF reported herein occur through an identical or parallel pathway.

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Address correspondence to P. G. Holt, Division of Cell Biology, Western Australian Research Institute for Child Health, Roberts Road, Subiaco, Western Australia 6008, Australia

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