

Original article

Role of cysteinyl leukotrienes in human allergen-specific Th2 responses induced by granulocyte macrophage-colony stimulating factor

Background: The pro-inflammatory cytokine, granulocyte macrophage-colony stimulating factor (GM-CSF), which is elevated in the lungs of atopic asthmatic patients, has been shown to enhance major histocompatibility class II expression of alveolar macrophages (AM). We hypothesized that exposure of AM and monocytes from atopic asthmatic patients to GM-CSF would enhance their antigen presenting function, and investigated putative mechanisms for this effect.

Methods: Alveolar macrophages were purified from bronchoalveolar lavage by plastic adherence. Monocytes and CD4⁺ T cells were purified from peripheral blood by magnetic bead separation. Antigen-presenting cell (APC) were pre-treated with GM-CSF, pulsed with allergen and cocultured with autologous T cells. T-cell proliferation was determined by tritiated thymidine incorporation and cytokine production by enzyme-linked immunosorbent assay.

Results: Exposure of allergen-pulsed AM and peripheral blood monocytes to GM-CSF significantly increased allergen-specific T-cell proliferation and T helper 2 (Th2) cytokine production. The enhanced response was dependent on costimulation by CD86, but not CD80. Inhibition of the 5-lipoxygenase pathway abrogated GM-CSF-mediated upregulation by monocytes of allergen-specific interleukin-5 (IL-5) and IL-13 cytokine production. Blocking of the cysteinyl leukotriene receptor 1 (cysLT₁) receptor by a specific receptor antagonist inhibited allergen-specific IL-5 production in response to GM-CSF pretreatment.

Conclusion: Exposure to GM-CSF enhanced the capacity of human APC from atopic asthmatic patients to induce allergen-specific Th2 responses by a mechanism involving cysLT. Novel immunotherapies, targeting production of GM-CSF or its actions on APC have the potential, therefore, to prove beneficial in treatment of patients with inflammatory airway disease.

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Immune responses are tightly controlled within the lung. Alveolar macrophages (AM) are located in the air-tissue interface and are proposed to play a key role in maintaining the immune homeostasis of the lung through their phagocytic and potent microbicidal activity (1, 2). In addition, AMs from normal healthy individuals are weak antigen-presenting cells (APC) for T-cell activation (2–4)

and adoptive transfer of AM reportedly abrogated hyperresponsiveness to allergic challenge in the murine lung, indicating a role in immunosuppression (5, 6).

Antigen-presenting cells are critical for antigen-specific T-cell activation and therefore changes in resident APC function are likely to precede or parallel changes in T-cell activation in asthma. A series of reports show that AMs from atopic asthmatic patients lose their immunosuppressive phenotype and demonstrate enhanced capacity to activate autologous T-cell proliferation and cytokine secretion (7–9).

A number of studies suggest a role for granulocyte macrophage-colony stimulating factor (GM-CSF) in regulating immune activation in the lung. First, GM-CSF expression is greatly increased in AM in asthma (10). Interferon- γ (IFN- γ), in contrast, which activates antimicrobial and tumoricidal properties of AM is not

Abbreviations: 5-LO, 5-lipoxygenase; AM, alveolar macrophage; APC, antigen-presenting cell; BAL, bronchoalveolar lavage fluid; cysLT, cysteinyl leukotriene; cysLT₁, cysteinyl leukotriene receptor 1; DC, dendritic cells; Dex, dexamethasone; FCS, foetal calf serum; GM-CSF, granulocyte macrophage-colony stimulating factor; HBSS, Hanks-buffered salt solution; IFN, interferon; IL, interleukin; LT, leukotriene; LTB₄, leukotriene B₄; LTD₄, leukotriene D₄; mAb, monoclonal antibody; MFI, mean fluorescence intensity; PBM, peripheral blood monocytes.

increased (11). Our previous studies have demonstrated that GM-CSF, unlike IFN- γ , increases major histocompatibility complex class II antigen expression by AMs (12). Both dendritic cells (DC) and monocytes are recruited to the lung in disease, and we have previously shown that maturation of respiratory tract DC in the presence of GM-CSF induces potent T helper 2 (Th2) responses (13). Studies from animal models of atopic asthma also support a role for GM-CSF in regulating immune activation in the lung. Granulocyte macrophage-colony stimulating factor expression in the lung, following administration of an adenovirus vector, caused antigen-specific eosinophilia, and hyperresponsiveness (14). Bilyk and Holt (15) have demonstrated that GM-CSF reverses the immunosuppressive activity of rodent AMs. A potential mechanism for the immunostimulatory effect of GM-CSF was suggested by the observation that exposure of AM to GM-CSF stimulated production of the proinflammatory lipid mediators, cysteinyl leukotrienes (cysLT) (16). Moreover, GM-CSF knock-out mice demonstrated impaired production of cysLT by AM (17).

Cysteinyl leukotrienes, derived from arachidonic acid through the 5-lipoxygenase (5-LO) pathway, are increased following segmental allergen challenge in the lung (18). Cysteinyl leukotrienes have been demonstrated to induce changes relevant to allergen driven airway inflammation such as increased bronchial muscle contraction and vascular permeability (18). The cysLT receptor 1 (cysLT₁) is reportedly upregulated in cells present in the asthmatic airway (19). Further strong evidence of the role of cysLT in airway inflammation is that the inhibitor of the 5-LO pathway, zileuton, and antagonists of cysLT₁, inhibit the symptoms of allergic inflammation (18, 20). We present evidence that (i) GM-CSF upregulates the antigen presenting function of monocytes and AMs for proliferation and Th2 cytokine production; and (ii) cysLT are implicated in enhancing antigen presenting capacity by GM-CSF.

Materials and methods

Reagents

Interleukin-2 (IL-2) was purchased from Eurocetus (Harefield, Middx, UK). Other cytokines used for cell culture or enzyme-linked immunosorbent assay (ELISA) standards were purchased from R & D Systems (Abingdon, Oxon, UK). Dexamethasone (Dex) was obtained from Sigma Chemical Co (Poole, Dorset, UK). Fluorochrome-conjugated antibodies for flow cytometry, antibody-matched pairs for ELISA, and purified antibodies for cell culture were from Pharmingen/Becton-Dickinson (Cowley, Oxon, UK). Allergens for cell culture (cat dander, grass pollen mix, and house dust mite) were obtained from ALK Abello Ltd. (Hungerford, Berks, UK). Zileuton was provided by Abbott plc (Maidenhead, Berks, UK). The cysLT₁ antagonist, LY171883, was obtained from Cayman Chemicals (Tallinn, Estonia). RPMI, AIM-V medium, Hanks-buffered salt solution (HBSS), Dulbecco's phosphate buffer saline, and L-glutamine were purchased from Gibco Life Technol-

ogies (Paisley, UK). Foetal calf serum (FCS) was from PAA laboratories (Teddington, Middx, UK), and ciproxin from Bayer-Leverkusen (Leverkusen, Germany).

Subjects

This study was approved by the Guy's Research Ethics Committee and all subjects gave full written informed consent. Patients were recruited by advertisement and from the Asthma Clinic at Guy's Hospital, London. Asthmatic subjects were defined as having hyperreactive airways to histamine with a mean provocation concentration of histamine required to reduce forced expiratory volume in first second from baseline by 20% (PD20) of <1.0 μ mol. All asthmatic subjects fulfilled the American Thoracic Society criteria for mild asthma and were not taking steroids in any form for at least 2 weeks prior to study. Atopy was defined as the presence of a positive skin reaction (3 mm greater than the diluent control reaction) to at least one of a panel of aeroallergens. All volunteers were nonsmokers.

Bronchoalveolar lavage

Fibreoptic bronchoscopy was performed as previously described (10). A single 100 ml aliquot of warmed, normal saline was instilled into a subsegmental bronchus of the left side middle lobe and gentle suction was applied. The recovered bronchoalveolar lavage (BAL) fluid was centrifuged at 200 *g* for 10 min and the cell pellet washed three times in HBSS. A differential cell count was performed and cells cultured as previously described (10).

Cells

Fresh blood was obtained by venopuncture using sodium citrate (Sigma) as anti-coagulant. CD4⁺ T cells were isolated from washed whole blood by positive selection using Dynabeads and Detach-a-bead (Dyna, Wirral, Merseyside, UK) according to the manufacturer's instructions. Peripheral blood mononuclear cells were obtained by centrifugation over a Lymphoprep density gradient (Nycomed, Birmingham, UK) and monocytes were isolated using a negative selection monocyte isolation kit (Dyna). Flow cytometry (FACScalibur; Becton-Dickinson) was used to monitor the purity of the isolated cell populations and expression of costimulatory molecules.

Cell culture and assay

T cells were incubated in RPMI-1640 containing 2.5% of heat-inactivated autologous serum, 2 mM of L-glutamine, 100 ng/ml of ciproxin, and 0.2 U/ml of IL-2 at 37°C for 48 h prior to assay. Antigen-presenting cell populations were incubated for 48 h in flat-bottomed culture plates (Nunc; Gibco) in RPMI-1640 supplemented with 5% FCS, L-glutamine, and ciproxin with or without 50 ng/ml of GM-CSF alone or in combination with 10⁻¹⁰ to 10⁻⁷ M Dex. Interferon- γ was added at 50 ng/ml. The cells were then washed and pulsed with 1–50 μ g/ml of allergen for 2 h in 50% AIM-V medium containing 2.5% of serum, L-glutamine, and ciproxin. For cytokine detection, washed autologous T cells were added to the APC populations at a final concentration of 2×10^6 T cells/ml resulting in a final T cell : monocyte ratio of 3.3 : 1 and a T cell : AM ratio of 2 : 1, previously determined to be optimal for these assays, for 5 days. Where indicated, antibodies to CD80 or CD86, or zileuton, or the cysLT₁ antagonist were added to the

cultures at the same time as T-cell addition. For proliferation assays, cells were cultured for 7 days with addition of 0.66 μCi tritiated thymidine ($[^3\text{H}]\text{-TdR}$; Amersham International, Amersham, Bucks, UK) per plate for the final 18 h of culture. Proliferation, as correlated with $[^3\text{H}]\text{-TdR}$ incorporation was expressed as counts per minute $\pm$ standard error (SE).

ELISA

Cytokines were measured with commercially available matched antibody pairs according to the manufacturer's instructions. Lower limits of detection for these ELISA were: 200 pg/ml of IL-5; 100 pg/ml of IL-13; and 50 pg/ml of IFN- γ .

Statistics

The two-tailed *t* test was used to compare proliferation and cytokine production between groups.

Results

Exposure of alveolar macrophages to GM-CSF promotes antigen presenting function

The capacity of GM-CSF to enhance APC function by AM was investigated. Alveolar macrophage from five allergic asthmatic patients were preincubated in medium or with GM-CSF, and then pulsed with allergen to which the patient was sensitized. Allergen-pulsed AM preincubated with medium induced low levels of proliferation in autologous peripheral blood CD4^+ T cells (Fig. 1A). Following preincubation with GM-CSF, AM induced significantly increased ($P < 0.05$) proliferation of T cells at each concentration of allergen tested, with maximal effect (fivefold increase) observed at an allergen concentration of 10 $\mu\text{g}/\text{ml}$. The effect of the glucocorticoid, Dex, was then investigated. A concentration-dependent inhibition of proliferation was observed when GM-CSF pretreated and allergen pulsed AM were cocultured with T cells in the presence of Dex. The effect of pretreatment of allergen pulsed AM with GM-CSF on Th2 cytokine production was then assessed. Pretreatment of AM from two allergic asthmatic patients, sensitized to house dust mite or grass pollen, with GM-CSF enhanced subsequent IL-5 and IL-13 production by T cells (Fig. 1B). Production of IL-5 and IL-13 was enhanced three- to fourfold and two- to threefold, respectively. In contrast, exposure of AM to IFN- γ had no effect on IL-5 and IL-13 production in response to either allergen.

Exposure of peripheral blood monocytes to GM-CSF promotes allergen-specific Th2 cytokine responses

As it was possible to recover only a limited number of AM from BAL samples, the study was extended to investigate peripheral blood monocytes (PBM) from allergic asthmatics. Monocytes were purified from the peripheral blood of nine allergic asthmatics, pretreated

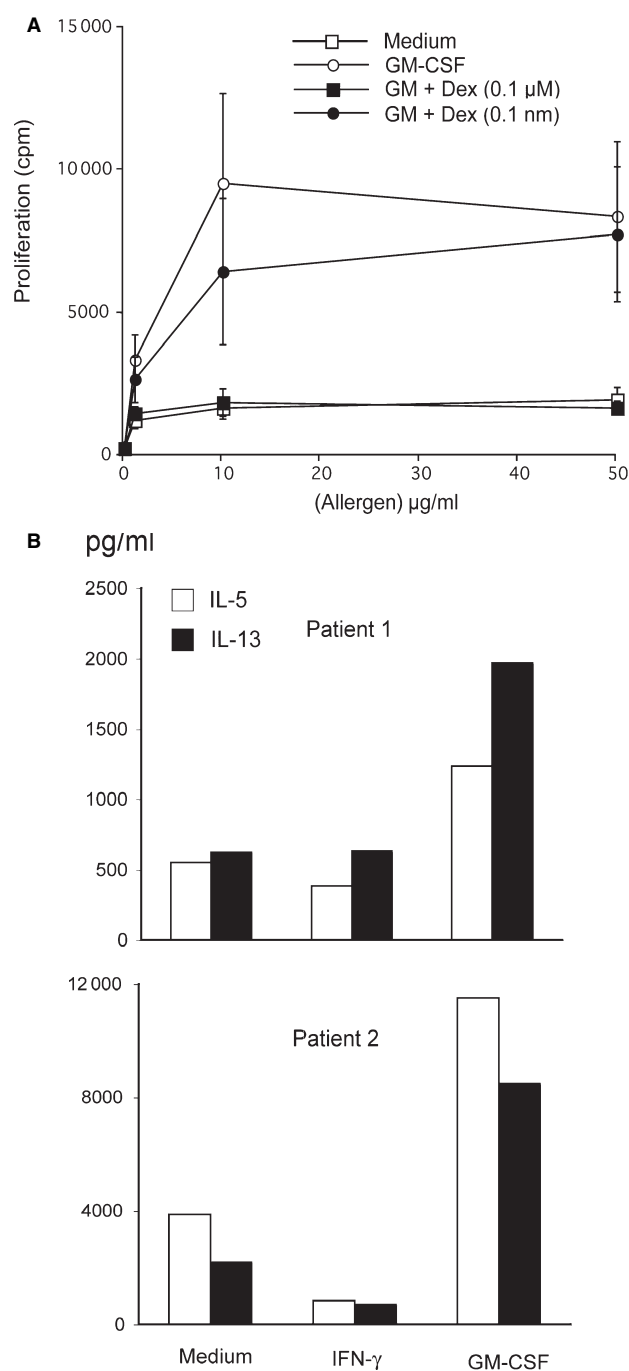


Figure 1. Pretreatment of alveolar macrophage (AM) with granulocyte macrophage-colony stimulating factor (GM-CSF) enhances their antigen presenting function. (A) AM were pretreated with medium alone, GM-CSF, or GM-CSF and dexamethasone (Dex), pulsed with allergen and cocultured with autologous CD4^+ T cells. Proliferation of T cells was determined after 7 days by $[^3\text{H}]\text{-TdR}$ incorporation. Results represent mean $\pm$ 1 SE ($n = 5$) (B) AM were pretreated with medium, interferon- γ (IFN- γ) or GM-CSF, pulsed with allergen and cocultured with T cells. Supernatants were harvested after 5 days and cytokine production determined by enzyme-linked immunosorbent assay (ELISA).

with medium or GM-CSF and pulsed with allergen. As we have previously reported (21) there was no difference between the viability of GM-CSF-treated and untreated monocytes following short-term incubation. In the absence of GM-CSF, allergen pulsed monocytes induced a low level of IL-5 and IL-13 production by autologous CD4⁺ T cells (Fig. 2). Pretreatment of monocytes with GM-CSF significantly enhanced ($P < 0.05$) both IL-5 and IL-13 production. In the absence of allergen, GM-CSF had no effect on cytokine production. There was a trend to increased IFN- γ production by allergen-specific T cells in response to GM-CSF treated monocytes, but this did not reach significance (mean + 1SE IFN- γ production in response to untreated and GM-CSF treated monocytes was, respectively, 573 + 331 and 1104 + 684 pg/ml). The addition of Dex (10^{-7} M) to the culture ablated Th2 cytokine production induced by GM-CSF pretreated monocytes. Corresponding to results with AM, pretreatment of monocytes with GM-CSF

significantly enhanced proliferation of autologous allergen-specific CD4⁺ T cells (data not shown).

Neutralization of the costimulatory molecule, CD86, abrogated Th2 cytokine production

Allergen-specific Th2 responses of bronchial cells from asthmatic patients are reportedly dependent on the principal costimulatory molecules, CD80, and CD86 (22). The effects of neutralization of CD80 and CD86 on monocyte induced Th2 production was, therefore, investigated. Addition of monoclonal antibody (mAb) to CD86 abrogated GM-CSF-mediated upregulation of IL-5 and IL-13 production by allergen-specific T cells from six atopic asthmatic patients (Fig. 3). The Th2 response was attenuated both in the presence and absence of GM-CSF, indicating the dependency of the T cell response on costimulation by CD86. In contrast, addition of mAb to CD80 or an isotype control antibody to the cultures had no effect on Th2 cytokine production.

A role for APC derived lipid mediators in promoting GM-CSF-mediated Th2 responses

The effect of the 5-LO inhibitor, zileuton, on induction of Th2 cytokine production by GM-CSF treated monocytes from six allergic asthmatic patients was investigated. Addition of zileuton, but not the vehicle control, to the assay caused concentration-dependent inhibition of IL-5 and IL-13 production by allergen-specific T cells (Fig. 4). Maximal inhibition in the assay was observed at a zileuton concentration of 10 μ M. A residual Th2 response was still detected in the presence of zileuton comparable with that observed with non-GM-CSF exposed monocytes.

As inhibition of the 5-LO pathway with zileuton abrogates production of both cysLT and leukotriene B₄, a potent chemotactic agent in the lung (23), the contribution of cysLT to GM-CSF-mediated upregulation of Th2 cytokine production was studied directly using a cysLT₁ antagonist. The cysLT₁ antagonist, LY171883, specifically inhibits binding of leukotriene D₄ (LTD₄) to the cysLT₁ (24). When the antagonist was added to cocultures of T cells and GM-CSF treated monocytes from two atopic asthmatic patients, concentration-dependent inhibition of allergen-specific IL-5 production was observed (Fig. 5). At a concentration of 10^{-6} M, LY171883 attenuated IL-5 production by T cells from both patients. The vehicle control had no effect on cytokine production.

The effect of the cysLT₁ antagonist on costimulatory molecule expression by PBM was then investigated. Exposure of PBM to GM-CSF for 48 h enhanced the level of expression of both CD86 and CD80. The mean fluorescence intensity (MFI) for CD86 expression increased from 276 to 569, while the MFI for CD80 increased from 16 to 28. The results are representative of

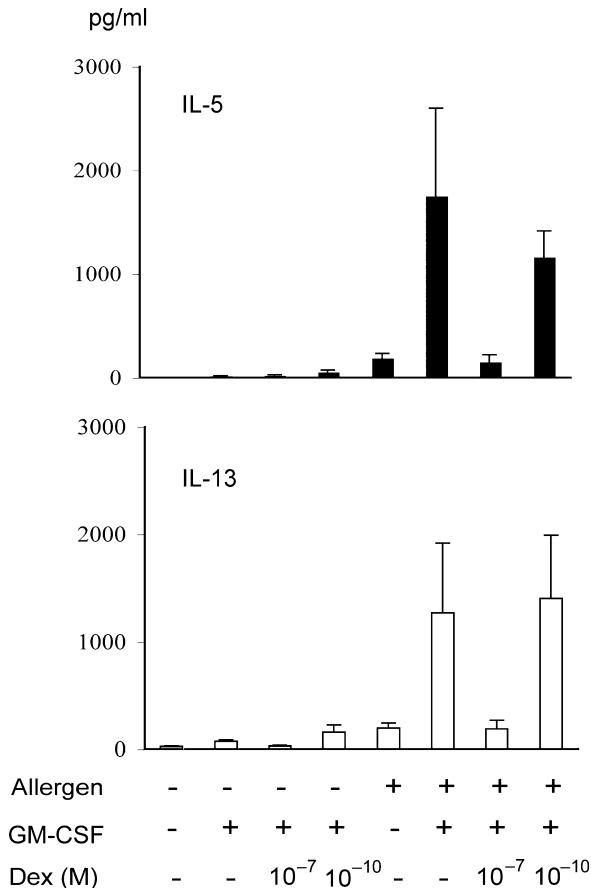


Figure 2. Pretreatment of monocytes with granulocyte macrophage-colony stimulating factor (GM-CSF) enhances allergen-specific Th2 responses. Monocytes were pretreated with medium alone, GM-CSF or GM-CSF and dexamethasone (Dex), pulsed with allergen and cocultured with autologous CD4⁺ T cells. Supernatants were harvested after 5 days and cytokine production determined. Results represent mean + 1SE ($n = 9$).

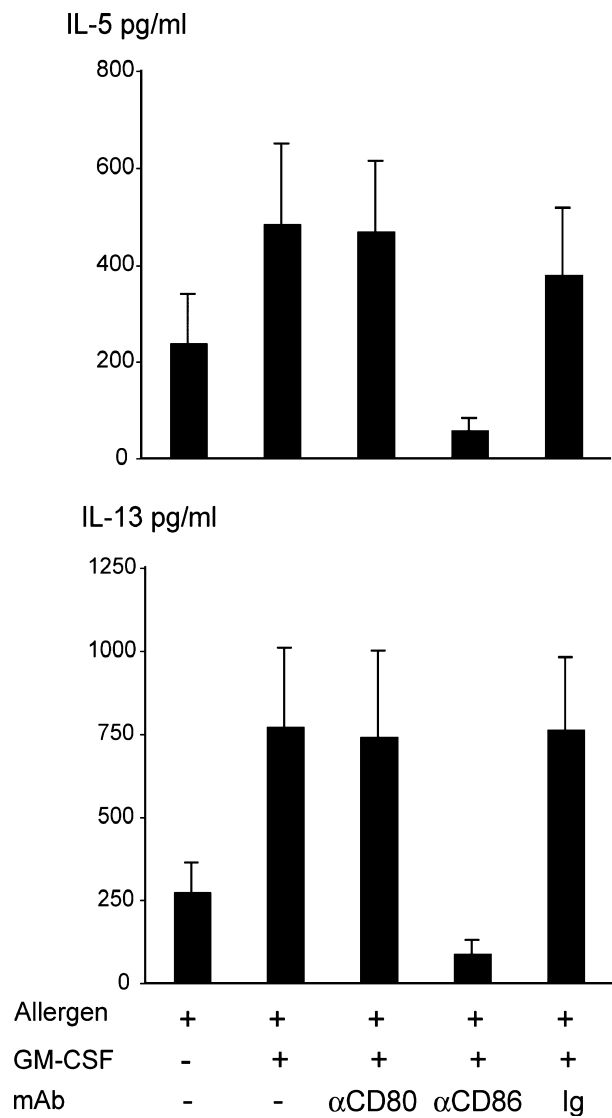


Figure 3. Antibodies to CD86 inhibit Th2 cytokine production by allergen-specific CD4⁺ T cells. Monocytes were pretreated with medium or granulocyte macrophage-colony stimulating factor (GM-CSF), pulsed with allergen and cocultured with autologous CD4⁺ T cells in the presence of antibodies to CD80 or CD86 or isotype control immunoglobulin (Ig). Supernatants were harvested after 5 days and cytokine production determined. Results represent mean + 1 SE (n = 6).

three separate experiments. Addition of the cysLT₁ antagonist to PBM at the same time as GM-CSF had no effect on expression of CD86 or CD80 (data not shown).

Discussion

The present study was designed to investigate potential mechanisms by which GM-CSF modulates the immunostimulatory phenotype of human APC. We have demon-

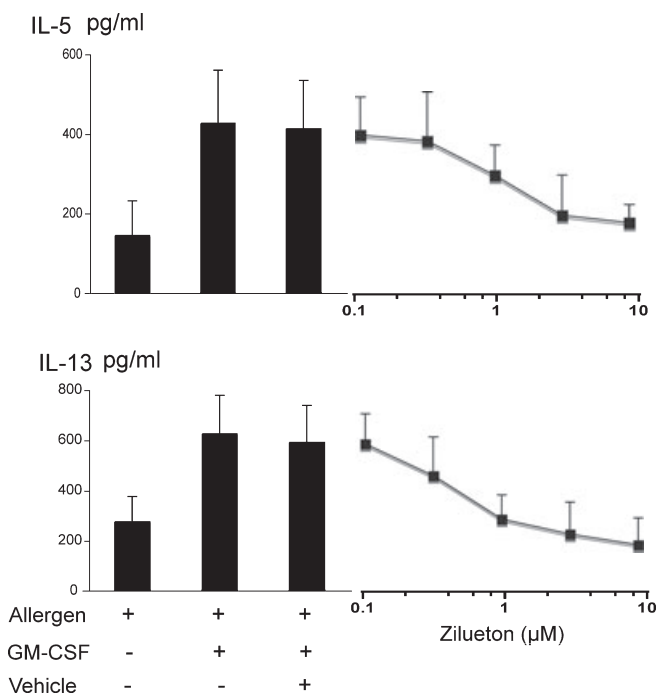


Figure 4. Zileuton inhibits allergen-specific Th2 responses induced by GM-CSF treated monocytes. Monocytes were pretreated with medium alone or granulocyte macrophage-colony stimulating factor (GM-CSF) and pulsed with allergen. Monocytes were then cocultured with autologous CD4⁺ T cells in the presence of varying concentrations of zileuton or vehicle alone. Supernatants were harvested after 5 days and cytokine production determined. Results represent mean + 1 SE (n = 6).

strated that the antigen presenting function of AM and monocytes from allergic asthmatic patients is upregulated on exposure to GM-CSF. Alveolar macrophages and monocytes pretreated with GM-CSF significantly enhanced proliferation of autologous allergen-specific CD4⁺ T cells and production of Th2 cytokines. The enhanced response to GM-CSF was shown to be inhibited by glucocorticoids, zileuton, and an antagonist of the cysLT₁.

Exposure to GM-CSF enhanced the antigen presenting function of AM but the limited numbers of AM recovered from asthmatic patients did not permit more extensive studies. Peripheral blood monocytes were, therefore, used as a model to investigate potential mechanisms of GM-CSF mediated immunomodulation. We have reported that PBM from atopic individuals express CD86 (21), a costimulatory molecule which we have shown to be upregulated on APC by GM-CSF (13). Initial experiments, therefore, examined the role of the principal costimulatory molecules, CD86, and CD80, in enhancing antigen presenting function. Neutralization of CD86, but not CD80, on GM-CSF pretreated monocytes ablated Th2 cytokine production by allergen-specific T cells. Both CD80 and CD86 have, however, been implicated as essential cofactors in IL-5 production in response to

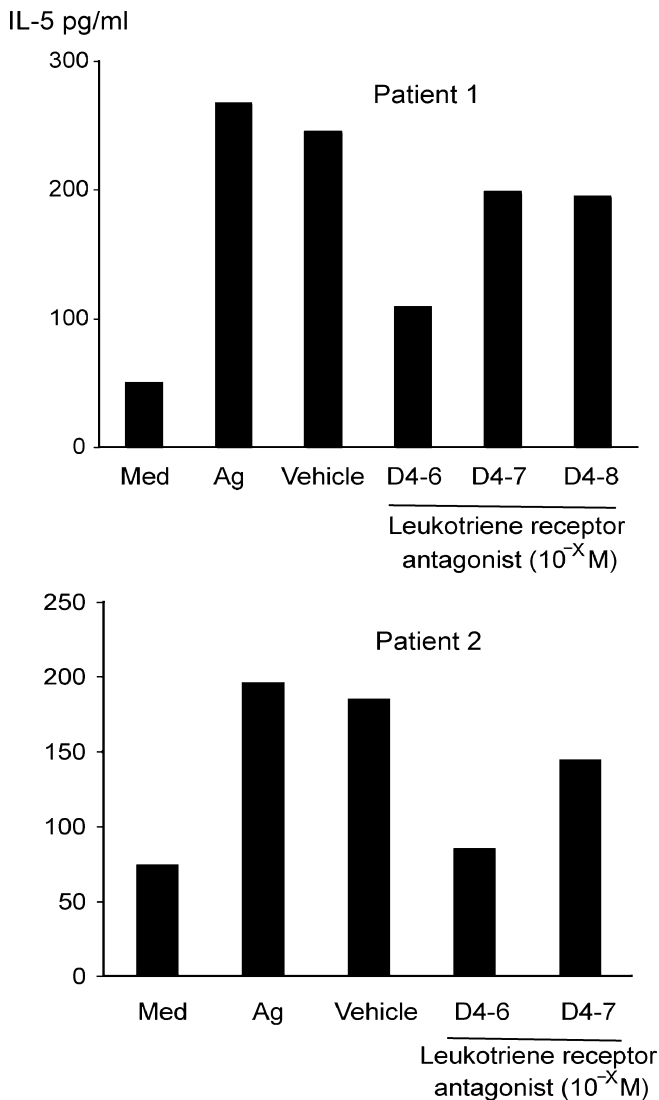


Figure 5. Inhibition of interleukin-5 (IL-5) production induced by granulocyte macrophage-colony stimulating factor (GM-CSF) treated monocytes by a cysteinyl leukotriene receptor 1 (cysLT₁) antagonist. Monocytes, obtained from two atopic asthmatic patients, were pretreated with medium alone or GM-CSF, and pulsed with allergen. Monocytes were then cocultured with autologous CD4⁺ T cells in the presence of varying concentrations of the cysLT₁ antagonist or vehicle alone. Supernatants were harvested after 5 days and cytokine production determined.

allergen stimulation of bronchial mucosal tissue from mild atopic asthmatics (22). Moreover, we previously reported that both CD80 and CD86 provided essential help for Th2 responses induced by respiratory tract DCs from patients with rhinosinusitis (13). These results indicate variable costimulatory requirements for induction of Th2 responses by disparate APC populations, perhaps resulting from the influence of environmental signals on maturation status. Indeed, exposure of PBM to

GM-CSF had a much greater effect on expression of CD86 than CD80.

Treatment of human immunodeficiency virus infected patients with GM-CSF has been reported to significantly enhance production of leukotriene (LT) (25). The possible involvement of LT in GM-CSF enhanced Th2 cytokine production was, therefore, investigated. Exposure of monocytes in the presence of GM-CSF to the 5-LO inhibitor, zileuton, abrogated IL-5, and IL-13 production by allergen-specific T cells, implicating LT in enhancing Th2 cytokine production. Although zileuton has been reported to inhibit IL-13 production by mitogen stimulated murine spleen cells (26), this is the first report that zileuton inhibits Th2 cytokine production by human allergen-specific T cells. This may be a specific effect on Th2 cytokine production as zileuton reportedly had no effect on proliferation by murine T cells (26).

A selective cysLTR₁ antagonist was found to inhibit Th2 cytokine production by allergen-specific T cells, indicating that Th2 cytokine production was dependent on the action of cysLT rather than other products of the 5-LO pathway. The antagonist has been shown to abrogate binding of LTD₄, the cysLT possessing the highest affinity for the cysLT₁ (27). The cysLT₁ is reportedly upregulated on human monocytes and macrophages following exposure to IL-4 or IL-13 (28), cytokines elevated in the asthmatic lung. Moreover, the cysLTR₁ is reportedly not expressed on T cells in the asthmatic bronchial mucosa (29). These data support the hypothesis that engagement of the cysLT₁, possibly in an autocrine fashion by APC, is an important component in amplification of Th2 responses by GM-CSF. The mechanism for this response is not, however, entirely clear as the cysLT₁ antagonist had no effect on upregulation of costimulatory molecules by GM-CSF. An animal model of allergic asthma has, however, implicated cysLT in promoting Th2 responses as a result of attenuated production by APC of IL-12, the prototype Th1-inducing cytokine (30).

Residual allergen-specific Th2 cytokine production was not inhibited by exposure to zileuton, suggesting that in the absence of GM-CSF, Th2 cytokine production was induced by a cysLT independent mechanism. Indeed, a recent report indicated that allergen-specific Th2 responses induced by monocyte-derived DC were not impaired by montelukast (31). It is thus probable that the induction and amplification of allergen-specific Th2 responses are dependent on both cysLT dependent and independent mechanisms, perhaps reflecting the origin of the APC and capacity to secrete cysLT. This may be a basis for the variability in the response of asthmatics to drugs which block cysLT production or the cysLT₁. Indeed, clinical experience as well as a formal trial (32) have demonstrated that the beneficial effects of zileuton were restricted to atopic asthmatic patients whose airways produced high levels of cysLT following allergen exposure.

These results provide the first evidence that exposure to GM-CSF enhances the capacity of human AM to induce allergen-specific Th2 responses. The results suggest a role both for upregulation of CD86 and an autocrine effect of LT in promoting immunomodulation by GM-CSF. Airway DC and AMs, are both believed to derive from monocyte precursors (33, 34) and are likely to be susceptible to similar mechanisms of immunomodulation.

Moreover, the fact that the cysLT_1 antagonist, montelukast, is steroid sparing in asthma (18) could reflect cooperative effects of steroids and cysLTR_1 antagonists in inhibiting GM-CSF induced antigen presentation. Novel immunotherapies, targeting production or the action of GM-CSF on respiratory cells have the potential, therefore, to prove beneficial in treatment of patients with chronic inflammatory airway disease.

References

1. Lambrecht BN. Alveolar macrophage in the driver's seat. *Immunity* 2006;**24**:366–368.
2. Lane SJ, Sousa AR, Lee TH. The role of the macrophage in asthma. *Allergy* 1994;**49**:201–209.
3. Gant VA, Shakoor Z, Barbosa IL, Hamblin AS. Normal and sarcoid alveolar macrophages differ in their ability to present antigen and to cluster with autologous lymphocytes. *Clin Exp Immunol* 1991;**86**:494–499.
4. Blumenthal RL, Campbell DE, Hwang P, DeKruyff RH, Frankel LR, Umetsu DT. Human alveolar macrophages induce functional inactivation in antigen-specific CD4 T cells. *J Allergy Clin Immunol* 2001;**107**:258–264.
5. Holt PG, Oliver J, Bilyk N, McMenamin C, McMenamin PG, Kraal G et al. Downregulation of the antigen presenting cell function(s) of pulmonary dendritic cells *in vivo* by resident alveolar macrophages. *J Exp Med* 1993;**177**:397–407.
6. Careau E, Bissonnette EY. Adoptive transfer of alveolar macrophages abrogates bronchial hyperresponsiveness. *Am J Respir Cell Mol Biol* 2004;**31**:22–27.
7. Larche M, Till SJ, Haselden BM, North J, Barkans J, Corrigan CJ et al. Costimulation through CD86 is involved in airway antigen-presenting cell and T cell responses to allergen in atopic asthmatics. *J Immunol* 1998;**161**:6375–6382.
8. Tang C, Rolland JM, Li X, Ward C, Bish R, Walters EH. Alveolar macrophages from atopic asthmatics, but not atopic nonasthmatics, enhance interleukin-5 production by CD4+ T cells. *Am J Respir Crit Care Med* 1998;**157**:1120–1126.
9. Lensmar C, Katchar K, Eklund A, Grunewald J, Wahlstrom J. Phenotypic analysis of alveolar macrophages and lymphocytes following allergen inhalation by atopic subjects with mild asthma. *Respir Med* 2006;**100**:918–925.
10. Hallsworth MP, Soh CP, Lane SJ, Arm JP, Lee TH. Selective enhancement of GM-CSF, TNF-alpha, IL-1 beta and IL-8 production by monocytes and macrophages of asthmatic subjects. *Eur Respir J* 1994;**7**:1096–1102.
11. Robinson D, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR. Activation of CD4+ T cells, increased TH2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. *J Allergy Clin Immunol* 1993;**92**:313–324.
12. Caulfield JJ, Fernandez MH, Sousa AR, Lane SJ, Lee TH, Hawrylowicz CM. Regulation of major histocompatibility complex class II antigens on human alveolar macrophages by granulocyte-macrophage colony-stimulating factor in the presence of glucocorticoids. *Immunology* 1999;**98**:104–110.
13. Faith A, McDonald J, Peek E, Richards D, Caulfield J, Chevetton E et al. Functional plasticity of human respiratory tract dendritic cells: GM-CSF enhances T(H)2 development. *J Allergy Clin Immunol* 2005;**116**:1136–1143.
14. Gajewska BU, Alvarez D, Vidric M, Goncharova S, Stampfli MR, Coyle AJ et al. Generation of experimental allergic airway inflammation in the absence of draining lymph nodes. *J Clin Invest* 2001;**108**:577–583.
15. Bilyk N, Holt PG. Inhibition of the immunosuppressive activity of resident pulmonary alveolar macrophages by granulocyte/macrophage colony-stimulating factor. *J Exp Med* 1993;**177**:1773–1777.
16. Brock TG, McNish RW, Coffey MJ, Ojo TC, Phare SM, Peters-Golden M. Effects of granulocyte-macrophage colony-stimulating factor on eicosanoid production by mononuclear phagocytes. *J Immunol* 1996;**156**:2522–2527.
17. Paine R III, Morris SB, Jin H, Wilcoxon SE, Phare SM, Moore BB et al. Impaired functional activity of alveolar macrophages from GM-CSF-deficient mice. *Am J Physiol Lung Cell Mol Physiol* 2001;**281**:L1210–L1218.
18. Holgate ST, Peters-Golden M, Panettieri RA, Henderson WR Jr. Roles of cysteinyl leukotrienes in airway inflammation, smooth muscle function, and remodeling. *J Allergy Clin Immunol* 2003;**111**:S18–S34.
19. Zhu J, Qiu YS, Figueroa DJ, Bandi V, Galczinski H, Hamada K et al. Localization and upregulation of cysteinyl leukotriene-1 receptor in asthmatic bronchial mucosa. *Am J Respir Cell Mol Biol* 2005;**33**:531–540.
20. Williams B, Noonan G, Reiss TF, Knorr B, Guerra J, White R et al. Long-term asthma control with oral montelukast and inhaled beclomethasone for adults and children 6 years and older. *Clin Exp Allergy* 2001;**31**:845–854.
21. Caulfield JJ, Hawrylowicz CM, Kemeny DM, Lee TH. GM-CSF increases the ability of cultured macrophages to support autologous CD4+ T-cell proliferation in response to *Dermatophagoides pteronyssinus* and PPD antigen. *Immunology* 1997;**92**:123–130.
22. Jaffar ZH, Stanciu L, Pandit A, Lordan J, Holgate ST, Roberts K. Essential role for both CD80 and CD86 costimulation, but not CD40 interactions, in allergen-induced Th2 cytokine production from asthmatic bronchial tissue: role for alphabeta, but not gammadelta, T cells. *J Immunol* 1999;**163**:6283–6291.
23. Del Prete A, Shao WH, Mitola S, Santoro G, Sozzani S, Haribabu B. Regulation of dendritic cell migration and adaptive immune response by leukotriene B4 receptors: a role for LTB4 in up-regulation of CCR7 expression and function. *Blood* 2007;**109**:626–631.

24. Fuller RW, Black PN, Dollery CT. Effect of the oral leukotriene D4 antagonist LY171883 on inhaled and intradermal challenge with antigen and leukotriene D4 in atopic subjects. *J Allergy Clin Immunol* 1989;**83**:939–944.
25. Coffey MJ, Phare SM, Cinti S, Peters-Golden M, Kazanjian PH. Granulocyte-macrophage colony-stimulating factor upregulates reduced 5-lipoxygenase metabolism in peripheral blood monocytes and neutrophils in acquired immunodeficiency syndrome. *Blood* 1999;**94**:3897–3905.
26. Kuvibidila S, Baliga BS, Gardner R, Yu L, Warrier R, Velez M et al. Differential effects of hydroxyurea and zileuton on interleukin-13 secretion by activated murine spleen cells: implication on the expression of vascular cell adhesion molecule-1 and vasoocclusion in sickle cell anemia. *Cytokine* 2005;**30**:213–218.
27. Lynch KR, O'Neill GP, Liu Q, Im DS, Sawyer N, Metters KM et al. Characterization of the human cysteinyl leukotriene CysLT1 receptor. *Nature* 1999;**399**:789–793.
28. Thivierge M, Stankova J, Rola-Pleszczynski M. IL-13 and IL-4 up-regulate cysteinyl leukotriene 1 receptor expression in human monocytes and macrophages. *J Immunol* 2001;**167**:2855–2860.
29. Zhu J, Qiu YS, Figueroa DJ, Bandi V, Galczenski H, Hamada K et al. Localization and upregulation of cysteinyl leukotriene-1 receptor in asthmatic bronchial mucosa. *Am J Respir Cell Mol Biol* 2005;**33**:531–540.
30. Okunishi K, Dohi M, Nakagome K, Tanaka R, Yamamoto K. A novel role of cysteinyl leukotrienes to promote dendritic cell activation in the antigen-induced immune responses in the lung. *J Immunol* 2004;**17**:6393–6402.
31. Saeki S, Matsuse H, Kondo Y, Machida I, Kawano T, Tomari S et al. Effects of antiasthmatic agents on the functions of peripheral blood monocyte-derived dendritic cells from atopic patients. *J Allergy Clin Immunol* 2004;**114**:538–544.
32. Hasday JD, Meltzer SS, Moore WC, Wisniewski P, Hebel JR, Lanni C et al. Anti-inflammatory effects of zileuton in a subpopulation of allergic asthmatics. *Am J Respir Crit Care Med* 2000;**161**:1229–1236.
33. Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity* 2003;**19**:71–82.
34. Slukvin II, Vodyanik MA, Thomson JA, Gumenyuk ME, Choi KD. Directed differentiation of human embryonic stem cells into functional dendritic cells through the myeloid pathway. *J Immunol* 2006;**176**:2924–2932.