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GM-CSF increases the ability of cultured macrophages to support autologous CD4$^+$ T-cell proliferation in response to *Dermatophagoides pteronyssinus* and PPD antigen

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SUMMARY

Previous studies have demonstrated an infiltration of monocytes and increased levels of granulocyte–macrophage colony-stimulating factor (GM-CSF) in the asthmatic lung. To study the possible effects of this cytokine upon the differentiation and function of these newly recruited monocytes, we have developed a model in which monocytes isolated from human peripheral blood were differentiated into macrophages in serum in the presence or absence of GM-CSF. After 7 days, the macrophages increased in size and granularity, had increased phagocytic activity, and expressed various adhesion molecules, CD14 and major histocompatibility complex (MHC) class II. The effects of GM-CSF on antigen presentation by cultured macrophages on the antigen-specific proliferative response of CD4$^+$ T cells to *Dermatophagoides pteronyssinus* or purified protein derivative of tuberculin and the mitogen phytohaemagglutinin was determined. CD4$^+$ T-cell proliferation was reduced when either antigen was presented by macrophages cultured in serum alone, compared with the values obtained with freshly isolated monocytes. However, CD4$^+$ cell proliferation was comparable to that observed with monocytes when antigen was presented by macrophages which had been pre-cultured with 50 U/ml GM-CSF. CD4$^+$ T-cell proliferation to phytohaemagglutinin was similar when all three populations were used as accessory cells. High numbers of macrophages partially suppressed CD4$^+$ T-cell proliferation in response to antigen presented by monocytes, but there was no significant difference between macrophages cultured in the presence or absence of GM-CSF. This data suggests that GM-CSF directs monocyte differentiation into macrophages with an antigen-presenting, rather than a suppressive, phenotype. Elevated levels of GM-CSF in the asthmatic lung may therefore maintain recently recruited monocytes in an inflammatory and T-cell activating state.

INTRODUCTION

Immunohistochemical analysis of bronchial biopsies obtained from patients with asthma have shown an augmented expression of granulocyte–macrophage colony-stimulating factor (GM-CSF), which is particularly prominent in the airway epithelial cells.[1] Additionally, stimulated monocytes from asthmatic subjects produce significantly more GM-CSF than those of non-asthmatic control individuals,[2] whilst alveolar macrophages (AM) obtained from bronchoalveolar lavage of asthmatic patients produce two- to threefold higher concentrations of GM-CSF as compared to normal, healthy volunteers.[3] GM-CSF directs the maturation and differentiation of macrophages and granulocytes,[4] increases eosinophil survival,[5,6] enhances the transmigration of eosinophils across endothelial cell monolayers and enhances expression of human leucocyte antigen D (HLA-D) molecules on antigen-presenting cells.[7] This cytokine may have an important role to play in the pathogenesis of the characteristic inflammatory responses in asthmatic airways.

Resident AM in healthy individuals are normally poor antigen-presenting cells and may even suppress CD4$^+$ T-cell proliferation.[8–10] However, in allergic asthma, the lymphostatic characteristic of AM is reduced relative to non-allergic controls[11] and this may reflect increasing numbers of infiltrating monocytes.[12] Monocytes are recruited into the airways in inflammation due, in part, to elevated levels of chemokines, such as monocyte chemoattract protein (MCP-1).[13]

Because these newly recruited monocytes may account for the increased antigen-presenting function observed in asthmatic bronchoalveolar macrophage populations, we wanted to determine how GM-CSF modulates the differentiation of these cells. We have developed an *in vitro* model in which peripheral blood monocytes were cultured in hydrophobic, Teflon bags for 7 days, based on a previously established

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technique, to obtain mature macrophages.[14] Using this model, we tested the hypothesis that GM-CSF modulates the differentiation of peripheral blood monocytes into an antigen presenting phenotype. Our results show that macrophages cultured in serum alone have decreased ability to support autologous $CD4^+$ T-cell proliferation in response to *Dermatophagoides pteronyssinus* and purified protein derivative of tuberculin (PPD), which is completely restored by preculturing the cells with GM-CSF.

MATERIALS AND METHODS

Monocyte isolation and culture

Venous blood was withdrawn from 13 volunteers, of which six were atopic, non-asthmatic individuals. Atopy was defined as the presence of at least two positive skin test reactions (3 mm greater than the diluent control reaction) to *D. pteronyssinus*. Peripheral blood mononuclear cells (PBMC) were isolated from whole blood and mixed 1:1 with Hank's balanced salt solution without Ca^{2+} or Mg^{2+} (HBSS) and centrifuged over a Lymphoprep (Nycomed, Oslo, Norway) gradient at 800 *g* for 20 min. Cells at the interface were collected and washed three times by centrifugation in HBSS. To remove B cells, the mononuclear cells obtained were resuspended in 1 ml phosphate-buffered saline (PBS) with 2% human AB serum (Sigma, Poole, UK) and incubated at 4° with anti-CD19-labelled Dynabeads (Dynal, 111.03, Wirral, UK), which had previously been washed three times in PBS/2% AB serum to remove sodium azide. After 30 min, the complex of B cells and Dynabeads were removed using a magnetic concentrator and remaining $CD19^-$ cells resuspended in RPMI at 4×10^6 cells/ml. Cells were seeded into serum-coated tissue culture flasks and incubated for 60 min at 37°. Non-adherent cells were removed by washing four times with HBSS and the adherent monocyte cell layer harvested by incubation with 3·3 mM ethylenediaminetetra-acetic acid (EDTA) for 20 min.

Monocytes were washed in HBSS with 5% AB serum by centrifugation, counted using a haemocytometer and viability assessed by trypan blue staining. Monocytes were resuspended in RPMI +5% AB serum, at a concentration of 2×10^6 cells/ml, in rectangular Teflon bags. Where indicated, 50 U/ml (5 ng/ml) recombinant human GM-CSF (R&D, Abingdon, UK) or a combination of 50 U/ml GM-CSF and 1000 U/ml interleukin-4 (IL-4) (R&D) were added to the cultures. Monocytes were incubated for 7 days at 37°.

Analysis of monocyte purity was assessed using a fluorescence-activated cell sorting (FACScan) flow cytometer (Becton Dickinson, Mountain View, CA,). Forward light scatter (FSC) and side light scatter (SSC) double parameter analysis showed that 90% cells fell within the monocyte gate, whilst 10% of cells were within the lymphocyte gate. The contaminating lymphocytes were $CD3^-$, $CD16^+$ and $CD56^+$ natural killer cells. No $CD19^+$ B lymphocytes were present.

Immunofluorescence labelling

The following panel of mouse anti-human antibodies were used to assess the purity and phenotype of the monocyte population. Anti-CD1a (NA1/34-HLK), anti-CD3 (UCH-T1), anti-CD4 (B-B14), anti-CD8 (B-H7), anti-CD11a (B-B15), anti-CD11b (44), anti-CD11c (3.9), anti-CD14 (UCH-M1), anti-CD16 (B-E16), anti-CD19 (B-C3), anti-CD54 (B-C14), anti-CD56 (B 159), anti-CD80 (BB-1), anti-HLA-DR (B-F1) and mouse immunoglobulin G_1 (IgG_1) – fluorescein isothiocyanate (FITC) and IgG_{2a} – phycoerythrin (PE) negative controls were purchased from Serotec (Kidlington, UK). Anti-CD86 (Bu63) was purchased from The Binding Site (Birmingham, UK).

For flow cytometric analysis, 1×10^5 cells were incubated with 10 µl of neat monoclonal antibody directly conjugated to either FITC or PE for 30 min at 4°. Cells were then washed by centrifugation in PBS/0·1% bovine serum albumin (BSA) at 200 *g* for 5 min and fixed in 1% paraformaldehyde. The percentage of cells labelled with each monoclonal antibody and the geometric mean of fluorescence intensity (MFI) was determined from single parameter histograms. The MFI value for immunoglobulin–FITC or immunoglobulin–PE was subtracted from the test value. To assess viability, 1×10^5 cells were incubated with 1 µl of 1 mg/ml propidium iodide (PI, Sigma) for 5 min at 4°, analysed immediately and cells which excluded propidium iodide were considered viable.

Phagocytosis assay

Freshly isolated monocytes or cultured macrophages were resuspended at 1×10^5 cells/ml RPMI +5% AB serum and incubated with 2×10^6 latex beads of diameter 2·97 µm (Sigma) at 37° for 60 min. As a control, cells were also cultured in the presence of 2×10^{-5} M Cytochalasin B (Sigma). After washing, cell suspensions were centrifuged onto single well slides (Hendley Ltd, Loughton, UK) at 19 *g* for 10 min using a cytocentrifuge (Shandon Inc., Pittsburg, PA) to give a final concentration of 2×10^4 cells per slide. Cells were then fixed in methanol, allowed to air dry, and stained with 5% Giemsa in Sörenson's buffer. One hundred cells were counted, and cells which had ingested five beads or more were scored as positive.

Lymphoproliferation assays

Monocytes were isolated from volunteers and the cells cultured for 7 days as described above. Six atopic donors were used in experiments in which *D. pteronyssinus* (house dust mite, HDM) was used as antigen, whilst these six atopic and seven additional non-atopic donors were used for experiments in which PPD was used.

On day 7, monocytes and $CD4^+$ T cells were isolated from the same donor. Lymphocytes were isolated by positive selection by incubation of the cells with anti-CD4-labelled Dynabeads (Dynal, 111.16) for 1 hr at 4°. The beads were washed four times in PBS/2% AB serum to remove non-specifically bound cells. $CD4^+$ T cells were then eluted from the beads by incubation with 100 µl Detach-a-bead (Dynal, 125.04) for 45 min and separated using a magnetic concentrator. The cells were then washed four times, counted and resuspended in RPMI +5% AB serum at a concentration of 1×10^6 cells/ml. The purity of $CD4^+$ T cells isolated were >99% $CD3^+$, $CD4^+$.

Either 1×10^4 monocytes or cultured macrophages and 1×10^5 $CD4^+$ T lymphocytes were added to flat-bottomed wells of 96-well microtitre plates (Nunc, Roskilde, Denmark). As controls, monocytes or $CD4^+$ T cells were cultured alone. Antigen (HDM or PPD), or the mitogen phytohaemagglutinin (PHA) were added at concentrations of 30 µg/ml and 100 µg/ml for HDM or 3 µg/ml and 10 µg/ml for PPD and

PHA. Each concentration of antigen was performed in triplicate. In some experiments, cells were set up with either 500 μM N^G-monomethyl-L-arginine (L-NMMA, Sigma) or 1×10^{-6} M indomethacin (Sigma). To study whether the macrophages were suppressive, either 5×10^4, 1×10^4 or 1×10^3 macrophages were added to triplicate wells containing 1×10^4 monocytes and 1×10^5 $CD4^+$ T cells and stimulated with PPD antigen. After six days, 0·5 μCi of [^{3}H]thymidine (Amersham International, Amersham, UK) was added to each well. After 18 hr, the cells were harvested onto glass fibre filter mats (Packard, Pangbourne, UK) and counted using a matrix 96 direct β-counter (Packard).

Statistics

Differences of antigen expression between monocytes and macrophages were compared using the Mann–Whitney test and the effects described are significant at $P < 0{\cdot}05$. Differences in $CD4^+$ T-cell proliferation in response to antigen and mitogen between monocytes and macrophages were compared by analysis of variance between the curves and the effects described are significant at $P < 0{\cdot}05$.

RESULTS

Monocyte yields and purity

The monocyte population (Fig. 1a) increased in size and granularity over 7 days in culture, in the absence (Fig. 1b) or presence of 50 U/ml GM-CSF (Fig. 1c). To assess cell viability, the monocyte and macrophage populations were gated on forward versus side scatter dot plots, and the percentage of non-viable (propidium iodide positive) cells were calculated on single parameter histograms for monocytes (Fig. 1d) and monocyte-derived macrophages (Fig. 1e,f). The percentage of cells staining positively for propidium iodide was <5% in all groups.

Monocytes expressed CD14, which increased on cells cultured with GM-CSF for 7 days (Table 1). To determine whether cells differentiated into a dendritic cell like phenotype, in some experiments monocytes were also cultured in 50 U/ml GM-CSF and 1000 U/ml IL-4, which directs the differentiation of peripheral blood monocytes into an immature dendritic cell phenotype.[15] Cells cultured with this combination of cytokines expressed much lower levels of CD14, compared to monocytes cultured in the absence of IL-4 (Table 1).

Phagocytosis assay

In a separate series of experiments, the ability of monocytes and macrophages to phagocytose latex beads was studied. In three separate experiments, the mean value of monocytes which had ingested five or more beads was 29%, which increased to 71% and 70%, respectively, for macrophages cultured with and without GM-CSF (Fig. 2). In the presence of the inhibitor, 2×10^{-5} M cytochalasin B, less than 2% of monocytes and macrophages ingested the latex beads.

Immunofluorescence labelling

Greater than 80% of monocytes expressed CD11a and HLA-DR, whilst greater than 60% expressed CD11b, CD11c, CD54 and CD14. After 7 days in culture in the presence or absence of GM-CSF, there was a significant decrease ($P < 0{\cdot}01$) in the percentage of macrophages which expressed CD11a, CD11b and HLA-DR, whilst there was no significant change in expression of CD11c, CD54 and CD14, compared to monocytes. Culturing macrophages with GM-CSF did not significantly change the percentage of cells expressing each receptor, compared to macrophages cultured in serum alone (Fig. 3a). After 7 days in culture there was a significant decrease ($P < 0{\cdot}05$) in the relative number of receptors per cell (as measured by MFI) of CD11a and CD11b expressed by macrophages cultured in serum alone compared with monocytes, whilst CD11b was decreased on macrophages cultured in GM-CSF.

Monocytes did not express CD80, whilst 20% expressed CD86 (data not shown). After 7 days in culture, less than 5% of macrophages cultured in serum alone expressed CD80, whilst 37% expressed CD86. The number of macrophages cultured with GM-CSF that expressed CD80 was 8% and that

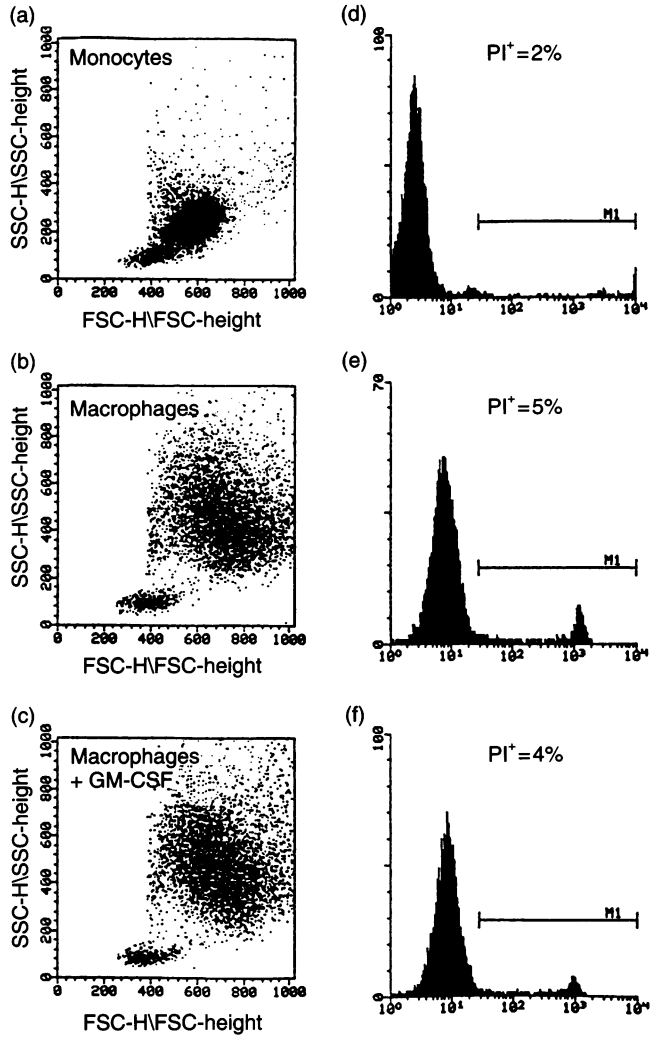

Figure 1. Double parameter histograms of FSC (size) versus SSC (granularity) of monocytes (a) and macrophages cultured in the absence (b) and presence (c) of 50 U/ml GM-CSF. The monocyte populations were gated, and single parameter histograms of propidium iodide positive cells (PI^+) was used to assess viability (d–f). Viabilities were >95%.

Table 1. Expression of CD14, CD80 and CD86 antigens on macrophages cultured for 7 days in serum only, serum + 50 U/ml GM-CSF or serum + 50 U/ml GM-CSF and 1000 U/ml IL-4. Data are presented as the mean fluorescence intensities of mean ± standard error of five experiments

Antigen	AB serum only	+50 U/ml GM-CSF	+50 U/ml GM-CSF +1000 U/ml IL-4
CD14	47±5	102±62	11±5
CD80	3±2	13±8	13±11
CD86	31±13	41±27	31±19

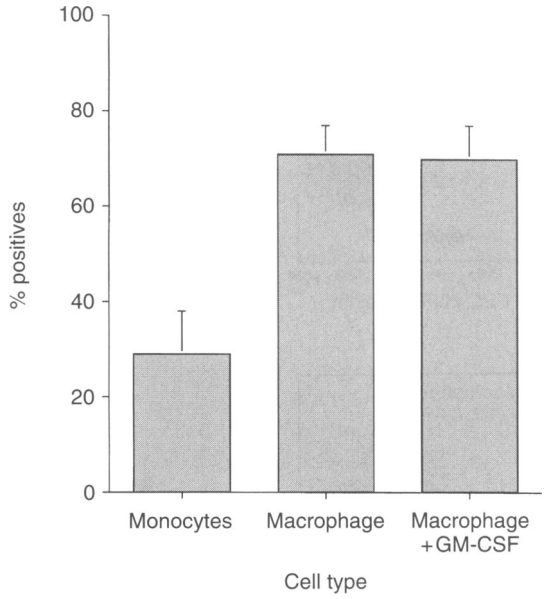

Figure 2. Comparison of the percentage of monocytes and macrophages which ingested five or more latex beads. Mean and SEM of three experiments.

expressed CD86 was 38%. However, receptor expression was slightly increased on macrophages cultured in GM-CSF (MFI = 41) compared to culturing in serum alone (MFI = 31) (Table 1).

Lymphoproliferation assays

The ability of monocytes and macrophages to process and present antigen to autologous $CD4^+$ T lymphocytes in response to recall antigen and allergen was determined by [^{3}H]thymidine incorporation. The values shown represent mean c.p.m. (±SEM) of the responses of 6, 13 and 8 individuals to HDM, PPD and PHA, respectively. Proliferation by $CD4^+$ T cells in response to HDM was significantly reduced ($P < 0.05$) when the allergen was presented by macrophages cultured in serum alone as compared to monocytes; mean c.p.m. were 5131 and 4950 for monocytes, and 2824 and 3067 for macrophages, at 30 μg/ml and 100 μg/ml HDM, respectively. Proliferation by $CD4^+$ T cells in response to HDM was significantly increased ($P < 0.05$) when the allergen was presented by macrophages which had been pre-cultured with GM-CSF, compared to macrophages cultured in its absence; mean c.p.m. were 4792 and 4898, respectively (Fig. 4a). In the control experiments, $CD4^+$ T cells cultured individually showed little proliferation (c.p.m. < 500). $CD4^+$ T-cell proliferation in response to PPD was significantly reduced ($P < 0.05$) when presented by serum-cultured macrophages as compared to monocytes; mean c.p.m. were 20 221 and 19 656 for monocytes as compared to 11 030 and 13 133 for macrophages at 3 μg/ml and 10 μg/ml PPD, respectively. Proliferation by $CD4^+$ T cells in response to PPD was significantly increased ($P < 0.05$) when the antigen was presented by macrophages which had been pre-cultured with GM-CSF, compared to macrophages cultured in its absence; mean c.p.m. were 20 178 and 19 875, respectively (Fig. 4b). Similar proliferative responses to PPD were observed with monocytes and macrophages isolated from atopic and non-atopic donors (data not shown). Responses to HDM were only observed in *D. pteryonssinus* allergic individuals. There were no significant differences in the $CD4^+$ T-cell proliferative responses to PHA in the presence of the three mononuclear phagocyte populations (Fig. 4c).

The effect of macrophages on $CD4^+$ T-cell proliferation to antigen presented by monocytes

To determine whether the reduced $CD4^+$ T-cell proliferation to antigen presented by macrophages cultured in serum alone was due to induction of a suppressive phenotype, macrophages were co-cultured with monocytes, $CD4^+$ T cells and antigen. $CD4^+$ T-cell proliferation in response to antigen presented by monocytes was reduced in the presence of 5×10^4 macrophages, cultured with or without GM-CSF (Fig. 5). At lower concentrations of these macrophages (1×10^4 and 1×10^3 (data not shown)), $CD4^+$ T-cell proliferation was comparable to that observed in the absence of macrophages.

To determine whether the decrease in $CD4^+$ T-cell proliferation observed was due to suppressive mediators released from macrophages, experiments were performed using inhibitors of nitric oxide (L-NMMA) and prostaglandin synthesis (indomethacin). In six experiments, $CD4^+$ T-cell proliferation to antigen presented by macrophages cultured in serum alone was not significantly ($P > 0.05$) increased in the presence of the nitric oxide inhibitor (c.p.m. = 9377 and 9589 for 3 μg/ml and 10 μg/ml PPD, respectively, for macrophages; c.p.m. = 9549 and 10 991 for macrophages + L-NMMA) or in five experiments with the PGE_2 inhibitor (c.p.m. = 12 377 and 14 291 for macrophages; c.p.m. = 12 549 and 11 991 for macrophages + indomethacin). Similarly, no significant increase was observed when either monocytes or macrophages cultured with GM-CSF were used as antigen-presenting cells (APC).

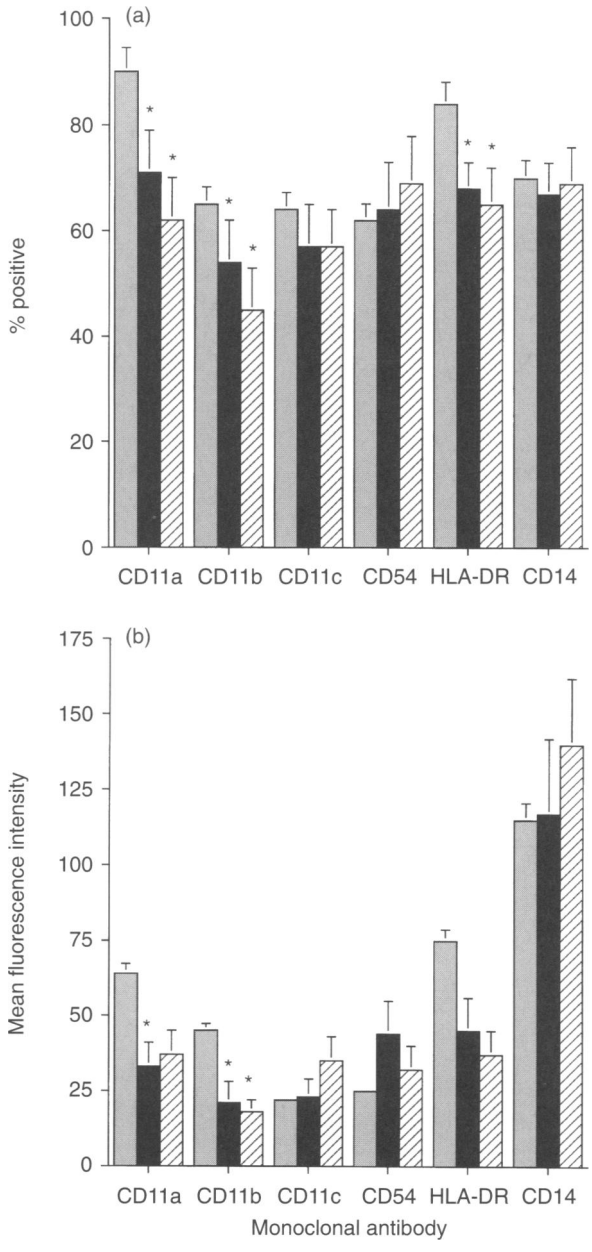

Figure 3. (a) The percentage of cells positive for each antibody was calculated from single parameter histograms for monocytes (▨), macrophages cultured with (▨) or without (■) GM-CSF. A histogram gate was set to exclude >95% of the control antibody, and the percentage of cells for the test antibody falling within this gate was determined. A significant ($P<0{\cdot}01$) decrease in the percentage of macrophages which were positive for each antigen, compared to monocytes, is denoted by *. The mean and SEM of 13 subjects is presented. (b) The number of surface receptors per cell was determined by calculating the geometric mean of fluorescence intensity (FI) of a single parameter histogram for each antibody for monocytes (▨), macrophages cultured with (▨) or without (■) GM-CSF. A significant ($P<0{\cdot}01$) decrease in antigen expression by macrophages, compared to monocytes, is denoted by *. The mean and SEM of 13 subjects is presented.

DISCUSSION

We have shown that culturing peripheral blood monocytes in the presence of serum leads to their differentiation into a cell phenotype with a reduced ability to support autologous $CD4^+$ T-cell proliferation in response to antigen and allergen. In comparison, addition of GM-CSF to these cultures maintains the cells in an antigen presenting phenotype.

Monocytes were isolated by positive selection of cells which had adhered to plastic tissue culture flasks. The initial isolation procedure contained many B cells, which also have the ability to present antigen in association with MHC class II. However, B cells were successfully removed using anti-CD19-coated magnetic beads. Monocytes constituted greater than 90% of the adherent cell population, of which >80% expressed the lipopolysaccharide (LPS) receptor, CD14. Cells were cultured in Teflon containers because they remain in suspension and are easy to recover. We used 50 U/ml GM-CSF to stimulate the cells because this concentration has previously been shown to promote cell survival and in its presence, monocytes differentiate into macrophages.[16]

After 7 days in culture, monocytes developed into macrophages on the criteria of expression of CD14, and an increase in size and phagocytic activity. To test whether dendritic cells were generated in culture, in five experiments adherent cells were also cultured in the presence of 50 U/ml GM-CSF and 1000 U/ml IL-4, and the expression of CD14 determined. This combination of cytokines have been shown to establish dendritic cells from blood mononuclear cells, which have the antigen-processing capacity of immature dendritic cells with a phenotype of $CD1a^{hi}$ and $CD14^{lo}$.[15] Macrophages cultured in GM-CSF expressed high levels of CD14, whilst cells cultured with GM-CSF and IL-4 had greatly reduced CD14 expression. In addition, there is evidence that 10% fetal calf serum is optimal for dendritic cell culture, whilst increasing concentrations of human serum (above 1%) is detrimental to the development of dendritic cells in mononuclear cell cultures.[17] These data suggest that our culture system predominantly generated macrophages, although this does not discount whether there were low numbers of contamining dendritic cells in the initial monocyte preparations, which are preferentially maintained in the presence of GM-CSF.

Alveolar and tissue macrophages have been reported to have an active phagocytic activity, although the percentage of alveolar macrophages capable of phagocytosis varies between investigators, with reports of between 40 to 80% of cells being positive, and appears to depend upon serum conditions and the nature of the target organism.[18] Pre-culture with GM-CSF had no effect upon the phagocytic ability of macrophages, although GM-CSF has been reported to up-regulate the low affinity Fc_γ receptor,[19] the low affinity Fc_ε receptor[20] and the complement receptor membrane attack complex 1 (MAC-1)/CR3 (CD11b),[19] which are involved in the uptake of antibody–antigen complexes. Therefore, macrophages are capable of uptake of antigen, and this is independent of GM-CSF.

We reasoned that one possible explanation for the decreased $CD4^+$ T-cell proliferation in response to antigen presented by macrophages cultured in serum alone may be due to an impaired ability to provide secondary signals to T lymphocytes. The interactions between CD80 (B7-1) and

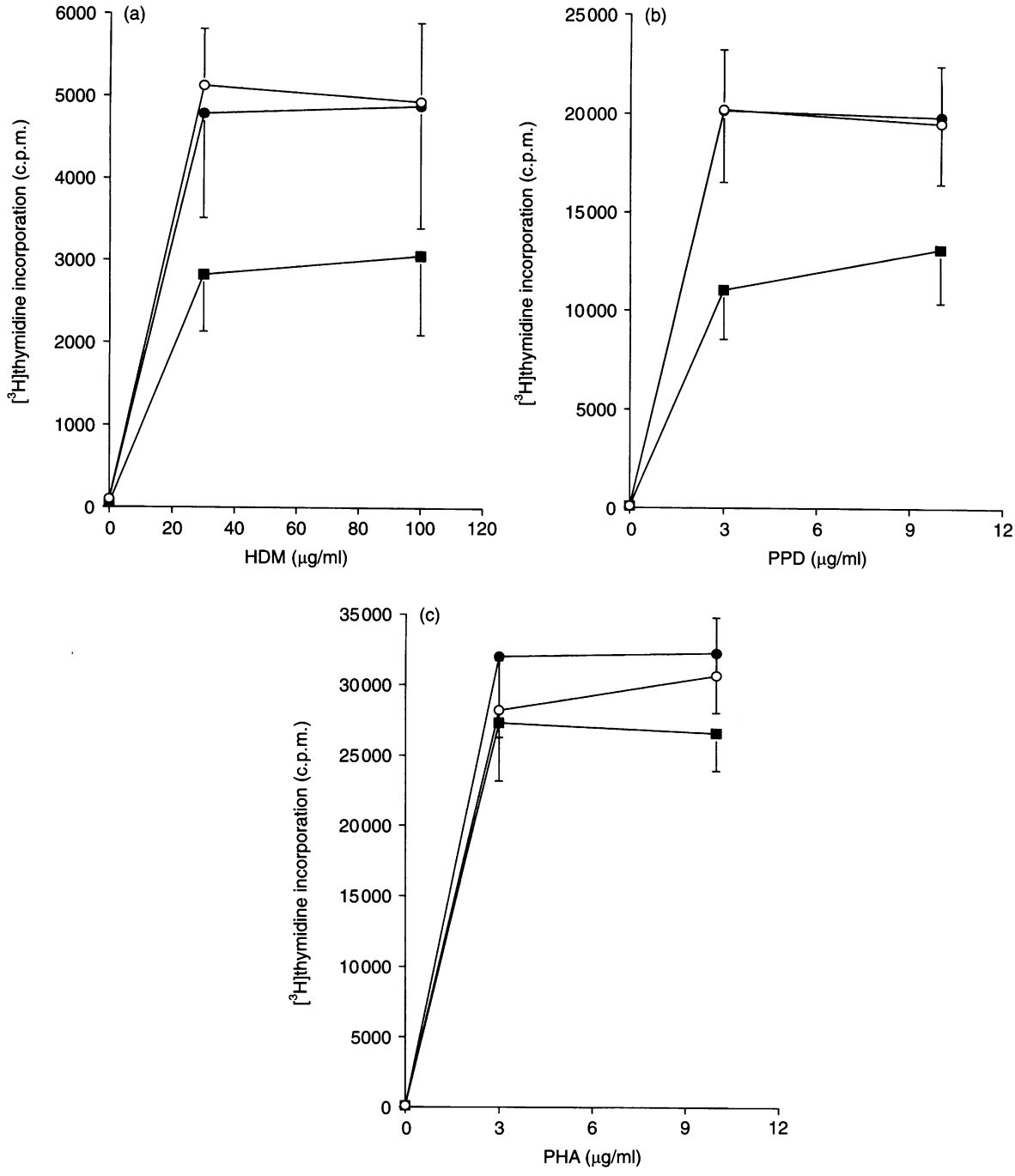

Figure 4. Autologous CD4$^+$ T-lymphocyte proliferation in response to HDM (a), PPD (b) or PHA (c) presented by monocytes (○), macrophages (■) or macrophages cultured in the presence of GM-CSF (●). Mean and SEM of six (HDM), 13 (PPD) and eight (PHA) experiments.

CD86 (B7-2) and their ligand CD28 is required for antigen driven T-lymphocyte proliferation.[21] Additional interactions are required, such as ligation of CD11a (leucocyte function-associated antigen; LFA-1) and CD54 (intracellular adhesion molecule 1; ICAM-1).[22] In this study, immunofluorescence labelling showed a significant decrease in the percentage of macrophages expressing CD11a, CD11b and HLA-DR after culture and expressed significantly reduced levels of CD11b when compared to monocytes. Macrophages cultured in serum only expressed significantly reduced levels of CD11a when compared to monocytes. GM-CSF has been reported to up-regulate MHC class II antigen expression,[23] although this induction is weak and requires synergy with the glucocorticoid agonist dexamethasone.[24] GM-CSF has been reported to induce CD86 on human peripheral blood monocytes, whilst this cytokine has no effect upon CD80.[25] Monocytes did not express CD80 and only low levels of CD86. GM-CSF-cultured macrophages had slightly enhanced levels of CD86 compared

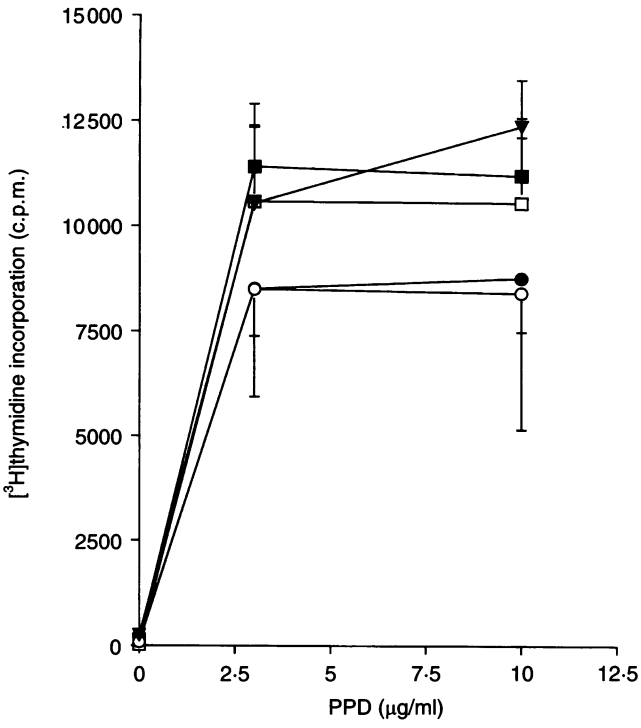

Figure 5. Autologous CD4$^+$ T lymphocyte proliferation in response to PPD antigen presented by 1×10^4 monocytes (▼), or monocytes plus 5×10^4 macrophages (○), 1×10^4 macrophages (□), 5×10^4 macrophages + GM-CSF (●) or 1×10^4 macrophages + GM-CSF (■). CD4$^+$ T-cell proliferation to PPD antigen presented by macrophages alone was 6156 and 7134 for 3 μg/ml and 10 μg/ml PPD, respectively, and macrophages + GM-CSF was 12013 and 11569, respectively. Mean and SEM of four experiments.

to macrophages cultured in serum alone, although it seems unlikely that the change is sufficiently great to account for the differences seen in APC function. CD4$^+$ T-cell responses to the mitogen PHA were not significantly different between the three populations of APCs. This finding suggests that macrophages express sufficient co-stimulation antigens to function as accessory cells for T cell proliferation. It is therefore unlikely that the changes in T-cell proliferation are due to differing expression of co-stimulatory molecules expressed by the APC populations.

In vitro tissue and alveolar macrophages have been reported to suppress antigen[26,27] and concanavalin A (Con A)-driven CD4$^+$ T-lymphocyte proliferation in rodent models,[28] although these lymphocytes express normal levels of CD3, T-cell receptor (TCR)αβ chains, up-regulate IL-2α and IL-2β receptors and exhibit an unimpaired Ca^{2+} flux.[29] Nitric oxide (NO^-) has been implicated as a macrophage derived T-cell suppressive molecule in rodents and its effects can be overcome by NO^- inhibitors.[30] AM have also been reported to have suppressive activity via prostaglandin E_2 (PGE_2) production,[31] which suppresses T-cell proliferation by inhibiting IL-2 production and down-regulation of the transferrin receptor.[32] Pretreatment of AM with cytokines derived from murine lung by LPS challenge attenuates their immunosuppressive activity and the major cytokine responsible for this is GM-CSF.[33] In this murine model, the reversal of the suppressive phenotype correlates with decreased NO^- generation.[34]

We therefore tested the hypothesis that reduced CD4$^+$ T-cell proliferation was due to suppression by macrophages and investigated the potential role of NO^- or PGE_2. Human AM derived T-cell suppression may be due to cell–cell contact[35] and large numbers of macrophages (ratio 0·5:1 for macrophages : T cells) partially suppressed CD4$^+$ T-cell proliferation to PPD antigen presented by monocytes. This effect was not observed when fewer macrophages were seeded into the monocyte cultures. This is similar to human AM, in which 50% inhibition of T-cell proliferation to mitogen is achieved only when there are equal numbers of macrophages to T cells and may represent a requirement for cell–cell contact.[36] Although high numbers of macrophages were suppressive, there was no difference in CD4$^+$ T-cell proliferation to antigen presented by monocytes in the presence of macrophages cultured with or without GM-CSF and addition of NO^- or PGE inhibitors to cultures did not increase CD4$^+$ T-cell proliferation to antigen presented by macrophages cultured in serum alone.

These results suggest that macrophages derived from human peripheral blood have a reduced antigen-presenting capability, rather than an increased suppressive capacity. These findings may have pathogenic implications for the characteristic inflammatory response in asthmatic airways. We suggest that monocyte recruitment through the asthmatic airway mucosa occurs via the secretion of cytokines, which include the chemoattractant MCP-1, and that these monocytes mature in the airways under the influence of GM-CSF into a phenotype which conserves their capacity to present antigen to CD4$^+$ T cells. This would be quite distinct from the situation in the healthy airways, in which the mature macrophages would differentiate into poor antigen presenting cells, which might indeed be suppressive for T-cell proliferation. The clinical implication of these findings is that the *in vivo* inflammatory processes in the asthmatic airways may be triggered by inappropriate presentation of inhaled allergens by freshly recruited monocytes which are maintained in a high antigen presenting phenotype by elevated levels of GM-CSF.

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