

Macrophages in chronic type 2 inflammation have a novel phenotype characterized by the abundant expression of *Ym1* and *Fizz1* that can be partly replicated in vitro

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Abstract

Using a murine model of nematode infection, we have discovered macrophages that display a novel phenotype that may be characteristic of macrophages in chronic type 2 inflammation. These nematode-elicited macrophages (NeM ϕ) are characterized by two unique features: the ability to actively suppress proliferation of a broad range of cell types and the high level expression of two novel macrophage genes, *Ym1* and *Fizz1*. NeM ϕ also show some similarities with in vitro-derived ‘alternatively activated macrophages’ such as the downregulation of inflammatory cytokines. We therefore investigated how much of the phenotype discovered in vivo could be replicated by activation with Th2 cytokines in vitro. *Fizz1* and *Ym1* were upregulated by IL-4 and IL-13 in vitro but at a considerably lower level than in NeM ϕ . In vitro treatment with IL-4 could also partly replicate the ability of NeM ϕ to block cellular proliferation. As well as the quantitative differences in gene expression and suppressive phenotype, we also observed phenotypic differences in the cell morphology between macrophages activated in vivo and in vitro. Although this study illustrated that macrophages activated in chronic inflammation have distinct features that cannot be readily reproduced in vitro it also demonstrated that some features of the complex NeM ϕ phenotype can be replicated by treatment of cultured macrophages with Th2 cytokines. In future, we hope to use in vitro analysis to help define the pathways that lead to this distinctive in vivo macrophage phenotype.

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1. Introduction

Macrophages display a wide variety of phenotypes depending on the cytokine environment, tissue localisation and the time point in the inflammatory process. In the event of an infection, the first population of macrophages recruited follow the well-defined classical activation pathway involving Toll receptor ligation and activation by Th1 cytokines such as IFN γ . Classically activated macrophages, in addition to their fundamental role in pathogen clearance, also secrete pro-inflammatory chemokines that further recruit other inflammatory effector cells to the site of infection [1]. Macrophages are also the key players in the resolution of inflammation [2] and under the influence of cytokines such as IL-4 and

IL-10 as well as prostaglandins and glucocorticoids, macrophages mediate apoptotic cell uptake, anti-inflammatory processes and wound healing [3–7]. Importantly, when inflammation is not resolved macrophages remain present as key elements in the chronic inflammatory pathway. However, whether these macrophages function to reduce inflammation and host damage or whether they contribute to host pathology is often uncertain.

We have been interested in addressing this question, particularly in the context of Th2 mediated inflammation such as that found in helminth infection, asthma and atherosclerosis. By using a murine model for nematode infection, we are able to obtain a source of macrophages activated in vivo in chronic Th2 inflammatory conditions [8]. Following peritoneal implant of the filarial nematode *Brugia malayi*, mice develop a potent Th2 response to the parasite [9] and maintain a long-term stable cellular infiltrate in the peritoneal

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cavity of which macrophages are the predominant cell type [10]. Using a combination of functional assays and gene expression analysis we have demonstrated that these nematode-elicited macrophages (NeM ϕ) have a novel phenotype characterized by two striking features: (1) the ability to reversibly suppress the proliferation of T cells and a wide range of tumour cell lines [8] and (2) the dramatic over representation of two gene products, *Ym1* and *Fizz1*, not previously associated with macrophage function [11].

Ym1, a member of the chitinase family, was originally described as an eosinophil chemotactic factor produced by CD8 lymphocytes [12]. A subsequent study has verified the eosinophil chemotactic ability of this molecule, both in vivo and in vitro [13]. We have found that *Ym1* represents 10% of the genes expressed by F4/80 purified NeM ϕ and that the level of expression is 10 000-fold higher than resident peritoneal macrophages [10,11]. In addition, one of the most striking features of *Ym1* is its propensity to produce large crystals in the lungs of mice with chronic lung pathology [14]. A molecule produced in such enormous quantities is unlikely to function solely as a chemotactic factor. One possibility is that *Ym1* is an effector molecule whose function may be to encapsulate chitin-bearing pathogens such as yeast, fungi or nematodes. Alternatively, Jin et al. [15] have suggested that it may function to interact with extracellular matrix components consistent with a role for Th2 driven macrophages in wound healing [5].

We found *Fizz1* through analysis of a subtractive library designed to define IL-4 dependent macrophage genes where it represented over 35% of the subtracted genes and 2% of the total NeM ϕ mRNA [11]. *Fizz1* was first identified in the lavage fluid of mice with experimentally induced asthma [16]. Subsequently, it has been recognized as a member of a family of secreted cysteine-rich molecules with similarity to resistin, a molecule produced by adipocytes which induces resistance to insulin [17]. Neither of these studies identified a role for macrophages or IL-4 in *Fizz1* production. A role for *Fizz1* in the inhibition of nerve growth factor (NGF) has been proposed [16] but its function in Th2 mediated settings such as allergy or helminth infection remains to be elucidated.

Because *Ym1* and *Fizz1* have been identified in several non-helminth settings and particularly in association with the lung, it suggests that the NeM ϕ phenotype we have described may be characteristic of macrophages associated with Th2 chronic inflammation and not restricted to either nematode infection or the peritoneal cavity. The phenotype of NeM ϕ , including suppression, downregulation of pro-inflammatory cytokines and the high level of expression of novel genes, is highly dependent on IL-4 in vivo [8,11,18]. We thus considered them to have an 'alternative activation'

phenotype, a term coined by Gordon et al. to distinguish macrophages activated by IL-4 or IL-13 from classically activated macrophages [19,20]. Alternatively activated macrophages (AAM ϕ) have subsequently been expanded to include the effects of IL-10 and glucocorticoids [21]. Until very recently, most studies of alternative activation have been carried out by in vitro treatment of cultured macrophages with Th2 cytokines and glucocorticoids [4,19,22–24]. NeM ϕ share some features of macrophages activated by Th2 cytokines in vitro such as the downregulation of inflammatory chemokines [11] and increased class II MHC expression (unpublished observation). We thus investigated whether any of the IL-4 dependent features of NeM ϕ we identified in vivo could be replicated in vitro. We demonstrate here that the NeM ϕ phenotype can be partly replicated in vitro, providing a powerful adjunct to in vivo studies for the elucidation of the function of these cells, which are central players in many chronic inflammatory situations.

2. Materials and methods

2.1. Generation of macrophages

For all experiments, mice used were 6–8-week-old male C57BL/6 or BALB/c.

In vivo-derived NeM ϕ : *B. malayi* adult parasites were removed from the peritoneal cavity of infected jirds purchased from TRS Laboratories (Athens, GA) or maintained in house. Mice were surgically implanted intraperitoneally (i.p.) with six live adult female *B. malayi*. Three weeks later the mice were euthanized by cardiac puncture and peritoneal exudate cells (PEC) were harvested by thorough washing of the peritoneal cavity with 15 ml of ice-cold media (RPMI) supplemented with 10% FCS.

In vitro-derived M ϕ : Thioglycollate-elicited macrophages were generated as described by Handel-Fernandez [25]. In brief, 0.8 ml of 4% thioglycollate medium brewer modified (Becton Dickinson) was injected i.p. into mice. Four days later, PEC were harvested, as above.

2.2. Purification of macrophages by adherence

The harvested PEC were plated in 24-well culture plates at 2×10^6 cells/well. Following 2–3 h adherence at 37 °C, the non-adherent cells were removed, leaving a cell population highly enriched for macrophages. To measure the macrophage purity, a sample of cells were removed from the plate by washing with 5 mM EDTA in warm PBS and analysed by FACS staining using the macrophage marker F4/80 (Caltag). Macrophage purity was estimated as > 85%.

2.3. Activation of *in vitro*-AAM ϕ

For the generation of *in vitro* AAM ϕ , thioglycollate elicited macrophages and J774 macrophages (ATCC Rockville, MD) were treated for 20 h with IL-4 (10 or 20 ng/ml) or IL-13 (10 ng/ml) (PharMingen) in complete medium (RPMI supplemented with 2 mM L-glutamine, 0.25 U/ml penicillin, 100 μ g/ml streptomycin and 10% FCS).

2.4. Photographs

For cell morphology photographs, cultured macrophages were allowed to adhere onto 22 \times 22 mm cover slips and fixed with 4% formaldehyde. Phase contrast photographs were taken using the 40 \times objective and a colour video camera (JVC) using the application software Openlab (3.0).

2.5. RNA extraction and real-time PCR

RNA was recovered from the cultured cells by direct addition of Trizol (GIBCO) into the wells. Total RNA was subsequently extracted according to the manufacturer's instructions. Following DNaseI treatment (Ambion) to remove contaminating genomic DNA, 1 μ g of RNA was used for the synthesis of cDNA using MMLV reverse transcriptase (Stratagene). β -actin expression of each cDNA sample was first quantified by real-time PCR and the samples adjusted to be equivalent for concentrations of β -actin. The standardized samples were then used for gene expression analysis of *Arginase1*, *Fizz1*, and *Ym1*. Relative quantification of the genes of interest was measured by real-time PCR, using the LightCycler (Roche Molecular Biochemicals). Five serial 1:4 dilutions of a positive control sample of cDNA were used as a standard curve in each reaction and the expression levels of the genes were estimated from the curve (arbitrary units). PCR amplifications were performed in 10 μ l, containing 1 μ l cDNA, 4 mM MgCl₂, 0.3 μ M primers and the LightCycler-DNA SYBR Green I mix. The amplification of β -Actin, *Fizz1* and *Arginase1* was performed under the following conditions: 30 s denaturation at 95 $^{\circ}$ C, 5 s annealing of primers at 55 $^{\circ}$ C and 12 s elongation at 72 $^{\circ}$ C, for 40–60 cycles. The fluorescent DNA binding dye SYBR Green was monitored after each cycle at 86 $^{\circ}$ C. For *Ym1* amplification, the annealing temperature was increased to 63 $^{\circ}$ C and the monitoring of SYBR Green fluorescence was performed at 85 $^{\circ}$ C. Additionally, the LightCycler PCR products were electrophoresed on 1% agarose gels and visualized by ethidium bromide staining. Primers for lightcycler PCR analysis were: β -Actin: TGGAATCCTGTGGCATCCATGAAAC and TAAAACGCAGCTCAGTAACAGTCCG. *Arginase1*: CAGAAGAATGGAAGAGTCAG and CAGATATG-

CAGG GAGTCACC. *Fizz1*: GGTCACAGTGCA-TATGGATGAGACCATAGA and CACCTCTTCACTCGAGGGACAGTTGGCAGC. *Ym1*: TCACAGGTCTGGCAATTCTTCTG and TTTGTCCTTAGGAGGGCTTCCTC.

2.6. Suppression assay

Suppression of proliferation caused by NeM ϕ and *in vitro*-derived AAM ϕ was measured as described previously [8]. In brief, macrophages from 100 μ l of PEC at 1 \times 10⁶ cells/ml were purified by adherence as described above, and then treated with IL-4 (10 ng/ml) for 20 h. The macrophages were then co-cultured with 1 \times 10⁴ EL-4 (ATCC) cells for 48 h. One microcurie of [³H]TdR in 10 μ l medium (RPMI) was then added to each well, and plates were incubated overnight before harvesting and counting using a liquid scintillation counter (Microbeta 1450, Trilux). Percent suppression was calculated in relation to the proliferation of the EL-4 cells (ATCC) co-cultured with untreated control macrophages.

3. Results

3.1. Macrophage alternative activation marker

Arginase1 is induced by *in vitro* treatment with IL-4 and in NeM ϕ

To date, the only well characterized marker of AAM ϕ is *Arginase1*, shown originally *in vitro* [26,27]. The hepatic isoform of Arginase (*Arginase1*) is the counterpart of inducible nitric oxide synthase (iNOS), which mediates the classical pathway of inflammatory macrophages, catalysing the conversion of L-arginine to nitric oxide [28]. *Arginase1* competes with iNOS and mediates the alternative metabolic pathway of L-arginine to L-ornithine and urea [29]. We have previously shown that NeM ϕ *Arginase1* expression *in vivo* is IL-4 dependent [11], consistent with studies of macrophages in the schistosome egg induced granuloma [5]. As a starting point in comparing NeM ϕ to *in vitro*-derived AAM ϕ generated by IL-4 treatment of thioglycollate-elicited macrophages, we directly compared *Arginase1* expression in these two macrophage populations. We also tested the mouse macrophage cell line J774 to see whether this established cell line could be used as a source of AAM ϕ . Quantification by real-time PCR showed that treatment of thioglycollate-elicited BALB/c macrophages with IL-4 resulted in a 7-fold increase in *Arginase1* expression, confirming their alternative activation (Fig. 1A). The upregulation of *Arginase1* was quantitatively similar to the *in vivo*-derived AAM ϕ (NeM ϕ). The murine macrophage tumour cell line J774 also followed the alternative activation pathway in

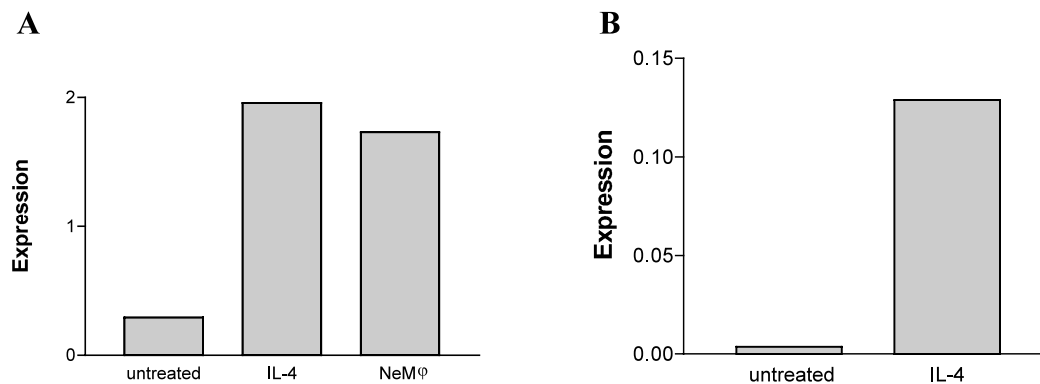


Fig. 1. The marker for alternative activation *Arginase1* is induced in both in vitro and in vivo-derived AAMφ (NeMφ). *Arginase1* expression is shown in untreated, IL-4 (10 ng/ml) treated thioglycollate-elicited macrophages and NeMφ (A) and untreated and IL-4 (10 ng/ml) treated J774 macrophages (B). Expression levels in arbitrary units were measured by real-time PCR using standard curves of five dilutions of cDNA template.

response to IL-4 and *Arginase1* expression was upregulated 36-fold (Fig. 1B).

3.2. Induction of *Fizz1* and *Yml* expression in response to IL-4

We have previously shown that *Fizz1* and *Yml* are the two most abundantly expressed genes in NeMφ [10,11]. We have also demonstrated a requirement for IL-4 for the in vivo induction of *Fizz1* but did not initially find *Yml* to be IL-4 dependent [10]. Subsequent analysis by others has found that *Yml* can be induced by both IL-4 and importantly IL-13 [30]. We have shown that a homologue of mammalian Macrophage Migration Inhibitory Factor produced by the parasite can directly induce *Yml* expression [10]. Thus, the lack of IL-4 dependence in our nematode implant model is likely due to the presence of IL-13 as well as the ability of the parasite to directly induce *Yml* expression.

As a *B. malayi* implant generates a strong Th2 response, we wanted to clarify the role of Th2 cytokines in the upregulation of these two novel genes. Direct induction of these genes in response to IL-4 in vitro would support the hypothesis that upregulation of *Fizz1* and *Yml* can be mediated by the Th2 cytokine environment generated by the parasite rather than by direct effects of the parasite itself. We therefore measured the expression of these two genes in IL-4 treated thioglycollate-elicited and J774 macrophages.

By real-time PCR we detected *Fizz1* in NeMφ at 25 amplification cycles, in IL-4 treated macrophages at 30 cycles and finally in untreated control macrophages at 45 cycles (Fig. 2A). Using a standard curve of five dilutions of *Fizz1* cDNA template, we quantified the expression levels of *Fizz1* (Fig. 2B). In comparison to control macrophages we estimated a 400-fold upregulation of *Fizz1* in IL-4 treated macrophages and 4000-fold upregulation in NeMφ. Using the same method, we estimated the upregulation of *Yml* to be 2000-fold in

IL-4 treated macrophages and 20000-fold in NeMφ (Fig. 2 D). The induction of both these genes by IL-4 confirmed their utility as molecular markers of AAMφ. Though exposure to IL-4 induced *Fizz1* and *Yml* gene expression both in vitro and in vivo, the upregulation of these genes was 10-fold higher in NeMφ than in in vitro-derived AAMφ.

Because J774 expressed *Arginase1*, we considered the possibility that these cells could be a convenient source of in vitro-derived AAMφ. However, we were unable to detect either *Fizz1* or *Yml* expression in IL-4 treated J774 macrophages. We did not pursue the study of IL-4 treated J774 macrophages as they showed only limited ability to respond to IL-4 in comparison to thioglycollate-elicited macrophages which were able at least in part to mimic the phenotype of NeMφ.

3.3. Induction of *Fizz1* and *Yml* expression in NeMφ can also be replicated by treatment with IL-13

The Th2 cytokine IL-13 shares the same receptor as IL-4 and has been shown to mimic the activity of IL-4 including the induction in vitro of the AAMφ phenotype [23]. We measured the upregulation of *Arginase1*, *Fizz1* and *Yml* in response to IL-13 and measured quantitatively the effects of this cytokine in comparison to IL-4. We showed by real-time PCR that IL-13 could upregulate all three macrophage genes in C57BL/6 thioglycollate-elicited macrophages, though the upregulation was somewhat lower in comparison to treatment with IL-4 (Fig. 3). This would imply that the effect of IL-13 alone would not account for the 10-fold higher expression of these genes in NeMφ.

3.4. IL-4 treatment of cultured macrophages can only partly replicate the suppressive phenotype of NeMφ

We chose next to determine whether the suppressive phenotype of NeMφ could be replicated by in vitro

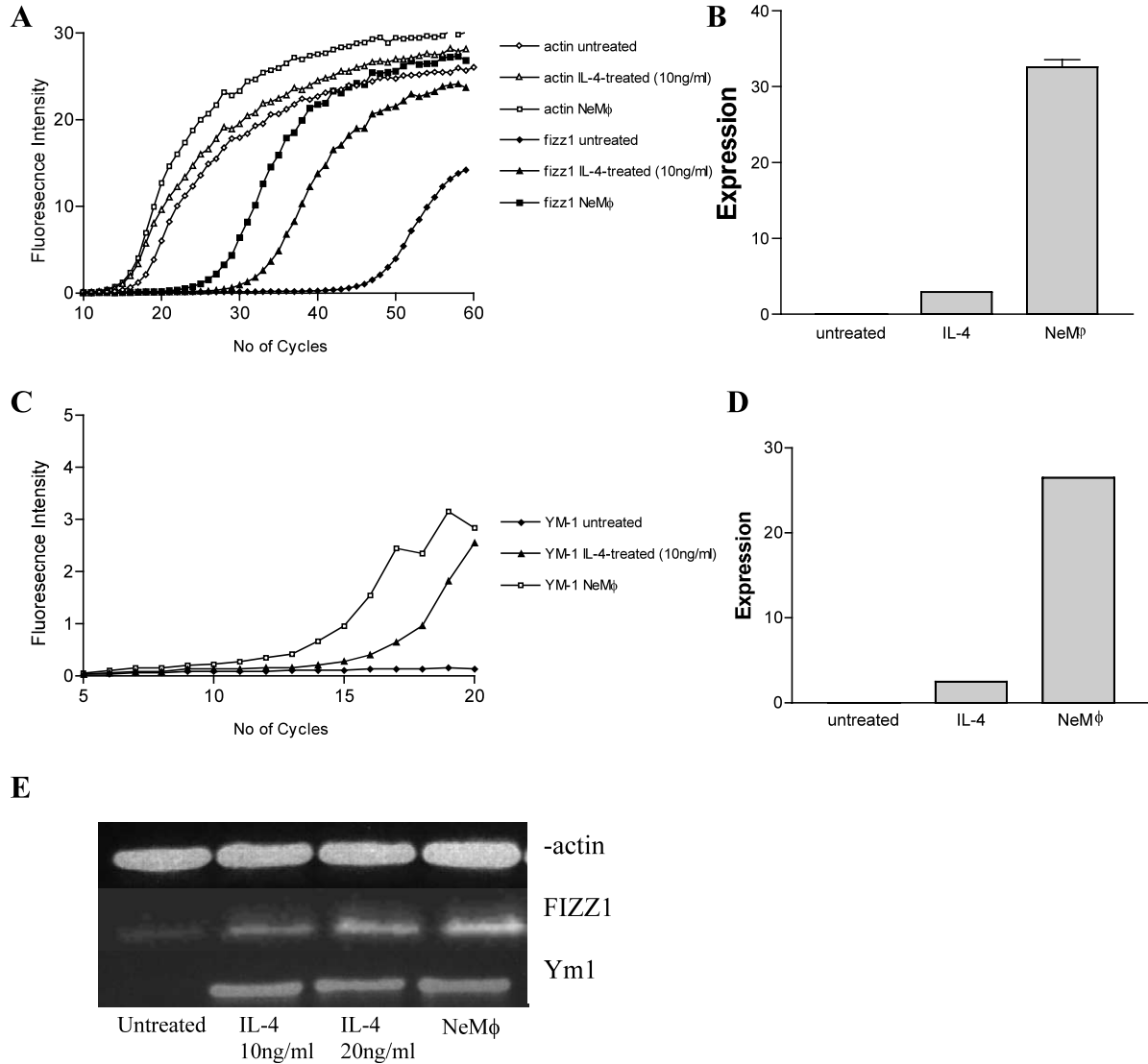


Fig. 2. *Fizz1* and *Yml* induction in NeM ϕ can be replicated in part by in vitro treatment with IL-4. Real-time PCR analysis shows the increase in fluorescence intensity during amplification of *Fizz1* and β -actin (A) and *Yml* (C). Expression levels of *Fizz1* (B) and *Yml* (D) in untreated, IL-4 (10 ng/ml) treated thioglycollate-elicited macrophages and NeM ϕ were quantified as described in Fig. 1. Real-time PCR samples were analysed on an agarose gel after the LightCycler run (E).

generation of AAM ϕ . We observed 62% suppression of proliferation of the EL-4 thymoma cells when co-cultured with IL-4 treated C57BL/6 thioglycollate-

elicited macrophages though this suppression was significantly lower than in co-culture with NeM ϕ (97%) (Fig. 4).

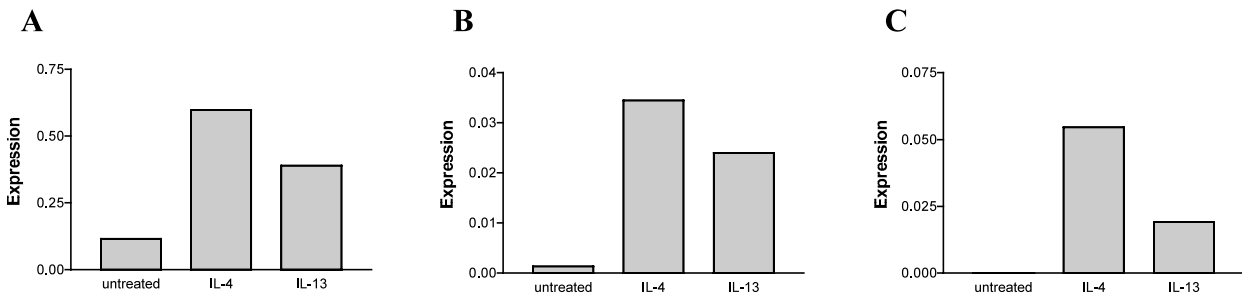


Fig. 3. IL-13 can also induce expression of *Arginase1*, *Fizz1* and *Yml*. Thioglycollate-elicited macrophages were treated with IL-4 or IL-13 (both at 10 ng/ml) and recovered for real-time PCR analysis of *Arginase1* (A), *Fizz1* (B) and *Yml* (C) expression. Expression level was quantified as described in Fig. 1.

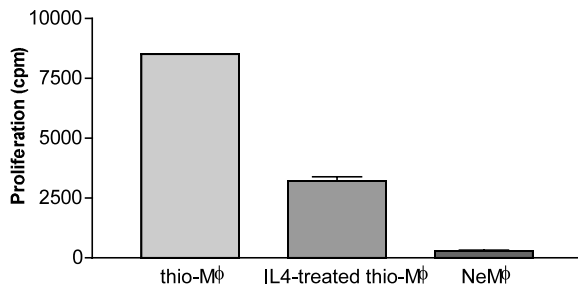


Fig. 4. IL-4 treatment can replicate in part the suppressive phenotype of NeMφ. Thioglycollate-elicited macrophages untreated and treated with IL-4 (10 ng/ml) and NeMφ were purified by adherence and co-cultured with the EL-4 thymoma cell line for 72 h and proliferation was measured by [³H] thymidine incorporation.

3.5. NeMφ and IL-4 treated macrophages have distinct cell morphology

Striking morphologic changes, including the spreading out and the tight adherence to the plate, have been reported when biogel-elicited macrophages are adhered overnight in the presence of IL-4 [19]. The effect of IL-4 on morphology is however strongly dependent on the type of macrophages used and how they are generated. Stein et al. for example observed no morphologic changes when thioglycollate-elicited macrophages were treated with IL-4 as the untreated control macrophages already adhered tightly to the plate [19]. In order to further characterize the relationship between in vitro and in vivo-derived AAMφ, we compared the morphology of NeMφ with thioglycollate-elicited macrophages treated with or without IL-4. Consistent with Stein's report, IL-4 treatment for 20 h did not noticeably change the morphology of the adhered thioglycollate-elicited macrophages and the macrophages looked tightly adherent with spread-out processes (Fig. 5A and B). To our surprise, NeMφ showed a distinct morphologic phenotype in comparison with the in vitro-derived AAMφ. They were less adherent, and

appeared more rounded, denser and less fibroblastic (Fig. 5C).

4. Discussion

We have used NeMφ as a powerful model for the study of alternate activation of macrophages in vivo particularly in relation to macrophages activated in type 2 chronic inflammatory settings such as asthma or nematode infection. In this study we determined the relevance of using in vitro treatment with type 2 cytokines as a means to investigate the function of these cells. In vitro activation of thioglycollate-elicited and J774 macrophages with IL-4 showed induction of the alternative activation marker *Arginase1* at a level comparable to NeMφ. *Fizz1* and *Ym1*, which we have identified as the most abundantly expressed genes in NeMφ [10,11] were also upregulated in the IL-4 treated thioglycollate-elicited macrophages but at a far lower level than in NeMφ. Further, the difference in cell morphology, which may imply a distinct functional role, and the lower suppressive capacity suggest that NeMφ have a distinct phenotype and function that can be replicated only in part by in vitro treatment with IL-4.

There are innumerable differences between in vitro and in vivo generation of AAMφ that could account for the higher expression of *Fizz1* and *Ym1* in NeMφ than in IL-4 treated thioglycollate-elicited macrophages. These include the synergistic effect of other cytokines such as IL-13 and the direct effect of parasite factors. For example, we have previously shown that the *B. malayi* secreted homologue of MIF can directly induce the expression of *Ym1* [10]. Further, the time frame and cellular context for the differentiation of the macrophages are considerably different. NeMφ are recruited and differentiated over 3–4 weeks in a chronic inflammatory setting in contrast with 20 h of in vitro IL-4 treatment of purified macrophages. Although the Th2 cytokine IL-13 was also able to induce expression of all

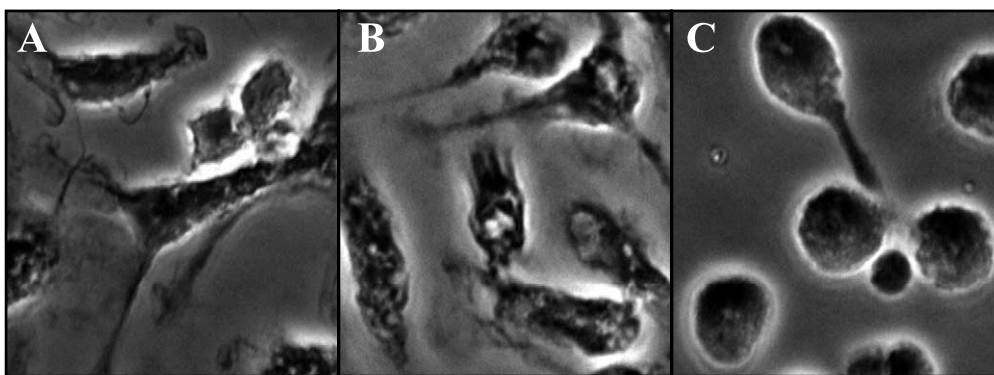


Fig. 5. In vitro and in vivo-derived macrophages have different cell morphology. Phase contrast micrographs were taken of untreated thioglycollate-elicited macrophages (A), thioglycollate elicited macrophages treated with IL-4 (10 ng/ml) for 20 h (B), and NeMφ, recovered 3 weeks post-implant and adhered overnight (C).

three genes, this alone cannot account for the in vitro and in vivo differences.

While this study was ongoing, two reports were published that described macrophages with alternatively activated features in the later stages of chronic parasite infection [31,32]. In both these reports, the phenotype of the macrophages changed over time, as the disease state became progressively more 'Th2'-like; emphasizing both the plasticity of macrophages and the complexity of defining macrophage function in vivo. In one study, Raes et al. also reported expression of *Fizz1* and *Ym1* in macrophages recruited during the chronic stages of infection in a murine model of trypanosomiasis [31]. Consistent with our findings, they demonstrated by conventional RT-PCR that these genes were induced by in vitro treatment with IL-4 and IL-13, indicating the importance of *Fizz1* and *Ym1* as molecular markers of AAM ϕ recruited during chronic Th2 inflammation. Our data confirms this report and further shows the quantitative differences between in vitro and in vivo-derived AAM ϕ . In addition, we observed that the tumour macrophage cell line J774, when treated with IL-4, upregulated *arginase1* but not *Fizz1* or *Ym1* expression, probably due to the lower plasticity of this highly differentiated cell line. This suggests that *Arginase1* may be a more general marker for activation by IL-4 whereas *Fizz1* and *Ym1* may be indicative of a distinct inflammatory phenotype. The functional importance of these molecules in chronic Th2 mediated inflammation, whether in promoting lung pathology or performing important immunoregulatory roles urgently needs to be addressed.

Although there is no evidence that either *Ym1* or *Fizz1* are involved in the suppressive capacity of NeM ϕ , our ability to induce expression of these genes, originally identified in vivo, by treatment with IL-4 suggested that it might be possible to similarly replicate in vitro the suppressive function of NeM ϕ . Treatment with IL-4 resulted in a moderate capacity to suppress proliferation of the EL-4 thymoma cell line (62%) though suppression was more profound with NeM ϕ (97%). The suppressive ability of macrophages activated under Th2 conditions has been observed before. Schebesch et al. showed that alternative activation of human macrophages in culture with IL-4 confers the capacity to suppress proliferation of PHA or ConA-stimulated CD4 T cells [22]. In both the in vivo-derived NeM ϕ and the in vitro treated human macrophages, suppression is not mediated by IL-10, prostaglandins or nitric oxide, and is not due to lack of co-stimulatory molecules [18,22,33]. Despite these similarities, we do not currently know whether the mechanism of suppression is identical in NeM ϕ and in vitro-derived human or mouse AAM ϕ . The main component of suppression in NeM ϕ is contact-dependent [8] although there may also be a secreted component that contributes to the suppression. The absence of

suppression as profound as that seen with NeM ϕ suggests that the contact-dependent suppression may not be fully replicated by in vitro-derived AAM ϕ .

It has recently been described that interstitial macrophages in the lung can also prevent cellular proliferation in a cell contact-dependent manner [34]. The mechanism of suppression bears striking similarity to the IL-4 dependent mechanism we have described for NeM ϕ . This emphasises the link with lung inflammation suggested by the high expression of *Fizz1* and *Ym1* in NeM ϕ , two genes that were originally identified in the context of chronic lung disease [14,16].

In addition to comparing gene expression and suppressive function of in vitro and in vivo-derived AAM ϕ , we also looked for similarities in cell morphology. We observed a striking difference in cellular morphology of in vitro AAM ϕ and NeM ϕ . Interestingly, the morphologic phenotype of NeM ϕ is surprisingly similar to macrophages treated with the synthetic glucocorticoid dexamethasone (Dx) [35]. Treatment with Dx causes alteration in the adhesion-signalling pathway involving paxillin and p130Cas, resulting in a change in macrophage cytoskeletal organisation. The Dx-treated macrophages are consequently more rounded and less adherent. This change in morphology is linked to the anti-inflammatory function of the macrophages, notably the increased phagocytosis of apoptotic neutrophils. This is consistent with our finding that NeM ϕ show increased phagocytosis of apoptotic neutrophils in comparison to thioglycollate-elicited macrophages (unpublished data). The presence of glucocorticoids is an additional in vivo factor that we have yet not replicated in vitro with IL-4. Though the literature on alternative activation of macrophages makes little distinction between activation by Th2 cytokines and by glucocorticoids [4,21], it is tempting to speculate that each have different effects on the morphology, migratory ability and anti-inflammatory function of macrophages. The role of macrophages activated by glucocorticoids in the resolution of inflammation is currently under investigation [6].

In vitro models are experimentally easier and often give more consistent results than in vivo studies. However it is important to consider the physiological significance of in vitro data and how applicable it is in vivo. In vivo, type 2 cytokines will be acting in concert with other mediators such as glucocorticoids and these factors will be acting at different points in the inflammatory process. Using a murine model of nematode infection, we have discovered macrophages activated in vivo that show an exciting new phenotype, but also have characteristics that define them as alternatively activated [11,36]. In this study we identified the common features shared by NeM ϕ and macrophages activated in vitro with IL-4. Our data demonstrates, not surprisingly, that the phenotype of NeM ϕ , developed in a very complex

chronic inflammatory setting, cannot be fully reproduced in vitro. However, there are enough similarities between NeM ϕ and in vitro-derived AAM ϕ that we could envisage using the in vitro model of alternative activation to understand more fully the contribution of individual cytokines, lipid mediators and glucocorticoids to the development and function of macrophages in chronic inflammation.

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