Immunology 1997 **90** 463–469

T-cell cytokines may control the balance of functionally distinct macrophage populations

V. J. TORMEY,* J. FAUL,† C. LEONARD,† C. M. BURKE,† A. DILMEC* & L. W. POULTER* *Departments of Clinical Immunology, Royal Free Hospital School of Medicine, London, UK, and †Respiratory Medicine, James Connolly Memorial Hospital, Blanchardstown, Dublin, Ireland

SUMMARY

As monocytes differentiate into mature macrophages, subsets emerge that exhibit stimulatory, suppressive or phagocytic potential. These functionally distinct subsets can be discriminated using monoclonal antibodies RFD1 and RFD7. As examples of all these subsets have been repeatedly identified within the macrophage pool in a variety of organs the overall functional capacity of this pool will depend on the relative balance of these subpopulations. This study investigates whether this balance present in mature macrophage populations can be regulated by the local influence of T-cell-derived cytokines. The dose-dependent effect of cytokines interferon-γ (IFN-γ), interleukins (IL) IL-2, IL-4 and IL-10 on the phenotype and function of monocyte-derived macrophages was determined. Subsets of mature cells were quantified by identifying $RFD1^+$ $RFD7^-$ stimulatory cells ($D1^+$); $RFD1^-$ $RFD7^+$ phagocytes ($D7^+$) and $RFD1^+$ $RFD7^+$ suppressive cells ($D1/D7^+$). IFN-γ and IL-4 increased the relative proportions of $D1^+$ stimulatory cells and upregulated HLA-DR expression. IFN-γ also increased the capacity of the mature macrophage pool to stimulate T-cell proliferation. IL-10 reduced the proportions of $D1^+$ stimulatory cells while promoting the differentiation of $D7^+$ phagocytes and $D1/D7^+$ suppressive cells. IL-10 induced changes also reduced the functional capacity of the mature populations to stimulate T cells in auto and allogenic mixed lymphocyte reactions (MLR). IL-2 had no effect on differentiation of monocytes. Thus IL-4 and IFN-γ are seen to induce the development of stimulatory macrophages while IL-10 promotes differentiation of monocytes to mature phagocytes and suppressive macrophages. It is concluded that mature macrophage phenotype is 'plastic' and under the control of T-cell-derived mediators. Furthermore, within the differentiating monocytes, phenotypic change appears to carry with it functional change, thus retaining the relationship between antigen expression and activity in the mature macrophage populations.

INTRODUCTION

Heterogeneity among normal macrophages is striking. As well as their recognized role as tissue phagocytes there are subsets of macrophages that induce T-cell responses (antigen presenting cells) and subsets with the capacity to suppress T-cell responses.[1,2] These populations may be phenotypically discriminated using the monoclonal antibodies (mAbs) RFD1 and RFD7.[3]

The mAb RFD7 recognizes a predominantly cytoplasmic antigen that is present in mature tissue phagocytes but absent on inductive antigen presenting cells such as veiled cells, Langerhan's cells and interdigitating cells.[4] RFD1 is a mAb that sees an epitope within the major histocompatability complex (MHC) class II complex with restricted expression to cells known to be stimulations of T cells.[4] These monoclonal antibodies have been used in this laboratory and by many other independent workers[5–8] to discriminate sub-populations of macrophages within the overall mononuclear phagocyte pool. Such studies revealed that one set of cells morphologically identifiable as macrophages expressed both antigens seen by these two mAbs.[9] These cells have been demonstrated *in vitro* to exhibit a suppressive function in relation to T-cell stimulation.[3,9,10] Within normal tissues a balance of these three sub-populations can be identified[11] and gross changes to this balance have been observed in a variety of clinical conditions.[12–16] With the existence of these functionally distinct populations it is clear that the overall capacity of the macrophage pool within any environment will be dependent on the balance of these three different types of cell. Thus regulation of this balance may be a crucial mechanism in sustaining homeostasis; and dysregulation may promote immunopathological dysfunction.

Received 24 September1996; revised 15 December 1996; accepted 16 December 1996.

Abbreviations: mAb, monoclonal antibody.

Correspondence: Professor L.W. Poulter, Department of Clinical Immunology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, UK.

© 1997 Blackwell Science Ltd

Many studies have revealed that cytokines released by T cells have a profound effect on macrophage function. On the one hand, cytokines such as interferon-γ (IFN-γ) have been repeatedly shown to induce macrophage activation in terms of increased microbicidal capacity,[17] and increased antigen presenting capacity,[18] while others such as interleukin-10 (IL-10) have been shown to inhibit pro-inflammatory cytokine production by macrophages[19,20] and reduce their antigen-presenting capacity by inhibiting co-stimulatory molecules.[21,22] Other studies have revealed that IL-4, another T-cell derived cytokine, is able to induce class II MHC expression on macrophages, but inhibits the production of other cytokines.[23,24] Together, these observations represent cogent evidence to support the hypothesis that the overall function of the macrophage pool may be regulated by signals coming from the T lymphocyte populations.

In inflammatory situations the macrophages present within tissue infiltrates are recruited as monocytes from the peripheral blood and mature to adult populations *in situ.* Aberations in the balance of these functionally distinct macrophage subsets that is demonstrated as occurring in a variety of pathological conditions[10–15] may be the result of phenotypic and functional changes to the mature populations, or the influence of locally produced T-cell cytokines on the differentiation of the recruited monocytes. This study investigates the effect of T-cell derived cytokines on the differentiation of peripheral blood monocytes in a controlled *in vitro* environment. It is revealed that different cytokines have profound yet distinct effects on the eventual balance of mature functionally distinct populations derived from a single monocyte pool. It is suggested that this regulation of monocyte differentiation may have a significant effect on those immunopathological situations where chronic inflammation is associated with dysregulation of T cell macrophage interaction.

MATERIALS AND METHODS

Monocyte harvest

Venous blood (30 ml) was drawn from healthy human adults in lithium heparin tubes. Mononuclear cells were separated by density centrifugation (Nycomed Pharma As, Oslo, Norway) at 650 ***g*** for 15 min. Mononuclear cells were washed with phosphate-buffered saline (PBS) three times and suspended at a density of 1×10^6 cells/ml in RPMI-1640 culture medium (Sigma-Aldrich Co. Ltd, Poole, UK) supplemented with 10% heat inactivated fetal bovine serum (FBS), 1·25% penicillin/streptomycin and 1·25% 200 mM glutamine. Aliquots (2 ml) were then transferred to each well of 24-well culture plates. The cultures were incubated at 37° in 5% CO_2 to separate monocytes by adherence. After 2 hr the non-adherent cells were removed by aspiration and each well was washed three times in PBS preheated to 37°. Fresh supplemented RPMI medium (2 ml) was then added to each well. For each culture experiment triplicate wells were harvested at this time (time zero = To). The method of harvest is described below. The cell populations at To contained consistently greater than 90% monocytes as determined by morphology; and viability (determined by trypan blue exclusion) was consistently greater than 95%.

Cell culture

Adherent monocytes were cultured in 24-well plates in supplemented RPMI (see above) for 7 days either with no addition or with the addition of cytokines. The cytokines used were recombinant IFN-γ, IL-2, IL-4 or IL-10; (all obtained from R&D systems, Abingdon, UK). These cytokines were added at different concentrations (5–20 ng/ml) and at different times during the 7 day culture period (see results). All were added in 20 μl aliquots with control cultures receiving 20 μl of sterile PBS. All solutions added were warmed to 37° before addition. Cultures were all harvested after 7 days. At time of harvest plates were placed at 4° for 30 min and then vigorously aspirated with fresh, cold PBS. All cells from the wells were collected including any cells no longer adhering to the plastic substrate. Cells were counted, viability was reassessed and only cultures with a viability greater than 90% were used for analysis. Experiments to determine dose response were performed twice. All other cultures were performed on nine separate occasions using samples from nine volunteers.

Cytospin preparation

After harvest at day 7 the cells were washed with PBS and centrifuged at 650 ***g*** for 5 min. The cell density was adjusted to $3–5 \times 10^5$ cells per ml and cytospins prepared by spinning 50 μl aliquots at 80 ***g*** for 2 min in a Shandon cytocentrifuge (Shandon Southern Products Ltd, Runcorn, UK). Cytospins were air dried for 1 hr and fixed in a 1 : 1 mixture of chloroform acetone for 10 min. These were then wrapped in cling film and stored at $-20°$ until analysed.

Immunocytological analysis

CD14 (UCHM1, University College Hospital school of Medicine)[25] and CD68 (EBM11, Dako Ltd, High Wycombe, UK)[26] expression were determined by indirect immunoperoxidase methods. The proportion of positive cells were counted using an Olympus microscope. The proportions of mature macrophage subsets within the harvested cell populations were determined by double immunofluorescence methods in which mAbs RFD1 (mouse immunoglobulin M (IgM)) and RFD7 (mouse IgGI (Royal Free Hospital School of Medicine, London, UK) were used in combination.[27] These reagents have been extensively used in this laboratory and by many independent workers to discriminate phenotypically distinct macrophage subsets. (They are commercially available from Serotec, Oxford, UK Ltd). By using two immunoglobulin class specific second layer reagents conjugated, respectively, to fluorescein isothiocyanate (FITC) and tetraethyl rhodamine isothiocyanate (TRITC) (Europath Ltd, Bude, UK), the relative proportions of $D1^+$ stimulating cells, $D7^+$ phagocytes, and $D1D7^+$ suppressive cells could be determined.[11] These proportions were quantified by counting multiple high powered fields using a Zeiss fluorescence microscope with epi-illumination and appropriate barrier filters for FITC and TRITC. For each subset the proportion of the cells was calculated by the formula:

$$\frac{\text{specific subset}}{D1^+ + D7^+ + D1D7^+} \times 100.$$

To determine the level of expression of MHC class II on the cultured cells the indirect immunoperoxidase method was applied using mAbs. RFDR1 (Royal Free Hospital School of

Medicine) which identifies a framework epitope on the HLA-DR molecule.[28] The level of HLA-DR expression was quantified by measuring the optical density of the peroxidase reaction product using a computerized image analyser (Seescan Imaging Ltd, Cambridge, UK).[29]

Mixed lymphocyte reactions (MLR)

In some experiments the peripheral blood monocytes cultured for 7 days in medium above alone or with IFN-γ or IL-10 added on day 5, were harvested and treated with mitomycin C.[14] Aliquots of 2×10^5 cells were introduced into 96-well microtitre plates and co-cultured with autologous or allogeneic T cells added to each well at a final concentration of 1×10^6 cells per well. This co-culture was incubated for 6 days. 18 hr before termination of cultures 1 μCi [^{3}H]thymidine was added to each well. Cells were harvested using an automatic cell harvester (Titer Tek; Flow Laboratories) and counted in a scintillation counter. The counts per minute expressed by the autologous cultures (without treatment of monocytes) were reduced to unity and all other cultures were expressed as a stimulation index calculated by the factor whereby this was greater than or less than the untreated autologous MLR (see results).

Statistical analysis

The effect of cytokine addition on macrophage phenotype (D1 and D7 expression) and HLA-DR expression was determined by analysis of variance (ANOVA). The ability of macrophage populations induced by IL-10 and IFN-γ to stimulate autologous and allogenic MLR was analysed using two-way ANOVA.

RESULTS

Monocytes exhibit phenotypic maturation in culture

At harvest after 2 hr adherence (To) over 90% of cells exhibit the morphological characteristics of monocytes with a cell count of 1×10^5 cells/ml. The majority expressed CD14 and CD68 antigens while only minimal numbers expressed the antigens seen by mAbs RFD1 or RFD7 (less than 3% in each case). No cells at this time exhibited the double phenotype D1/D7$^+$ (Fig. 1). The cell count remained at 1×10^5 cells/ml during the culture period with viability of 90%. No significant cell loss occurred over 7 days and no multiplication of cells

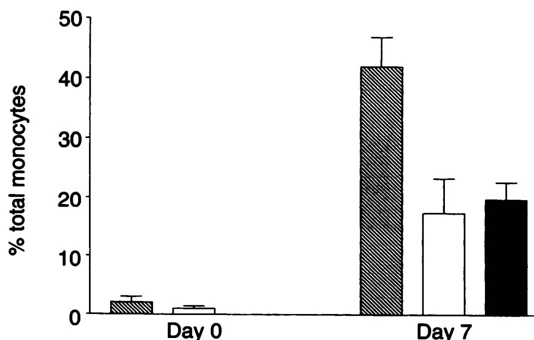

Figure 1. The proportions of monocytes exhibiting the stimulatory phenotype D1$^+$ (▨): phagocyte phenotype, D7$^+$ (□); and suppressive phenotype D1/D7$^+$ (■), after 2 hr adherence (day 0) and after 7 days culture (day 7). Mean ± SEM of nine experiments.

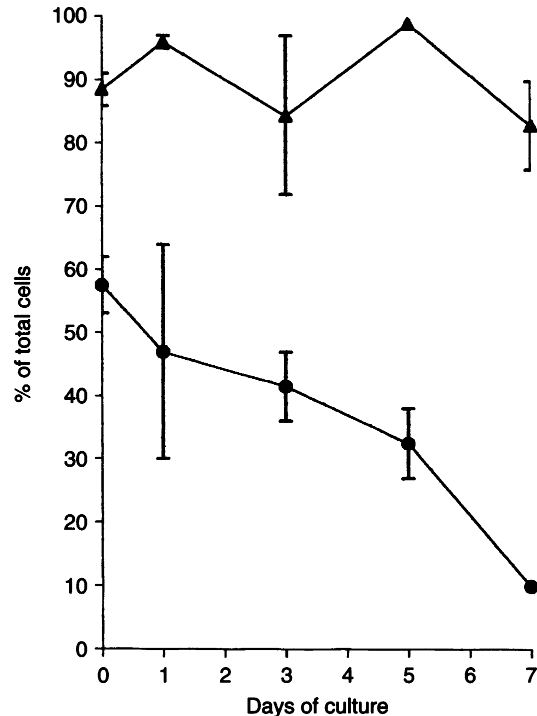

Figure 2. The percentage of total monocytes expressing CD68 (σ); and CD14 (●) by indirect immunoperoxidase methods at time 0 and after 1, 3, 5, and 7 days culture. Results represent mean ± SEM of two experiments.

was detected. After 7 days culture however, significant proportions of D1$^+$, D7$^+$ and D1/D7$^+$ cells were recorded. Approximately 80% of CD68$^+$ macrophages expressed one or both of the D1 and D7 antigens. A concurrent decline in the proportion of CD14$^+$ cells was observed (reduced to 10% of total) but no change in CD68 expression was seen (Fig. 2).

Contact with cytokine is most effective when added at day 5

A pilot study was designed to determine the most effective time for cytokine contact *in vitro*. IL-10 or IFN-γ each at a concentration of 10 ng/ml were added to monocytes cultures at progressive times during culture up to day 5. In all cases the cells were subsequently harvested for analysis at day 7. Both cytokines caused significant changes to the development of D1$^+$ and D7$^+$ cell proportions if added at the commencement of culture. Withholding IFN-γ addition until day 5, however, did not significantly alter the influence of this cytokine on phenotypic maturation whereas the effect of adding IL-10 at later times of culture resulted in a progressive reduction of D1$^+$ cells and a significant increase in D1/D7$^+$ cells (Fig. 3). As IFN-γ addition produced equivalent effects at all times of addition subsequent experiments used a 5-day time point for cytokine addition to give a standard time of 48 hr contact with all cytokines investigated.

T-helper type 1 (Th1)- and Th2-derived cytokines affect monocyte differentiation

Cytokines IL-10, IL-4, IL-2 and IFN-γ were added at various concentrations to 5 day monocyte cultures. All cells were

© 1997 Blackwell Science Ltd, *Immunology*, **90**, 463–469

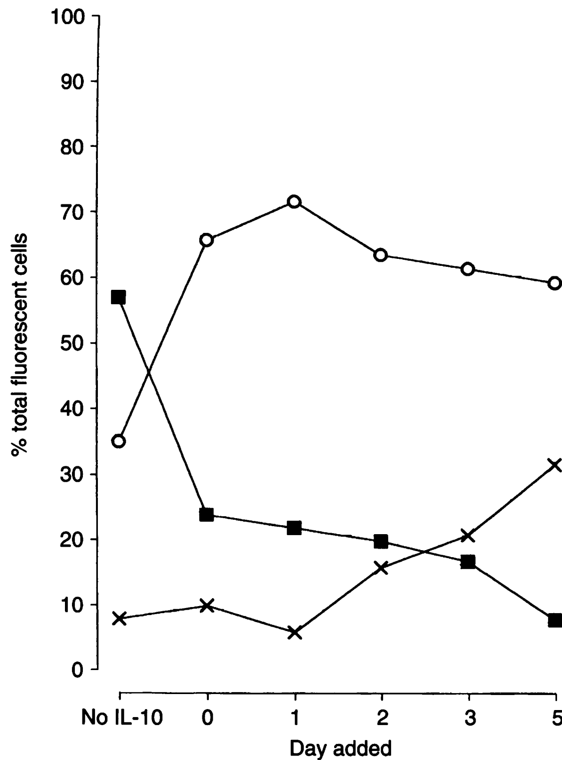

Figure 3. The effect on cell phenotype of adding IL-10 at time 0 (after harvest) or on days 1, 2, 3 or 5 of monocyte culture. Results represent the effect of cytokine addition on the relative proportions of Δ1$^+$ cells (■); D7$^+$ cells (○); and D1/D7$^+$ cells (×); quantified on day 7 of culture. Experiment performed in duplicate. Results of typical experiment shown.

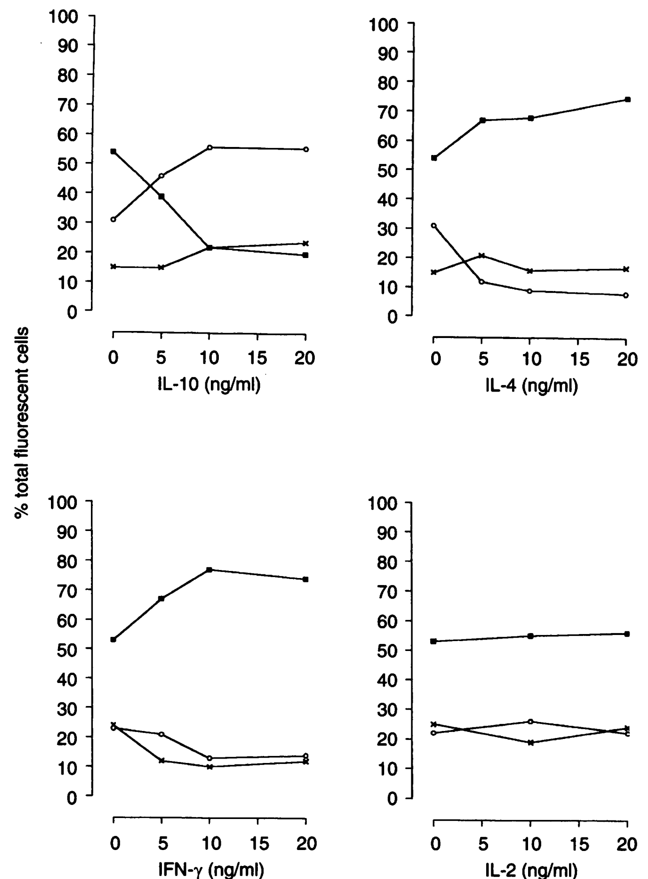

Figure 4. Dose–response curves demonstrating the effect of IL-10, IL-4, IFN-γ and IL-2 on the relative proportions of D1$^+$ (■); D7$^+$ (○) and D1/D7$^+$ cells (×) recorded after 7 days of culture. The different doses of each cytokine were all added on day 5. Experiment performed in duplicate. Results of typical experiment shown.

harvested at day 7 for analysis. IL-2 failed to affect the phenotype of differentiating monocytes at any concentration used (Fig. 4). IFN-γ and IL-4 were both effective in increasing the proportions of D1$^+$ cells while reducing proportions of cell expressing the D7$^+$ phenotype. IFN-γ was also seen to reduce the proportion of D1/D7$^+$ cells. Conversely, IL-10 significantly reduced the development of D1$^+$ cells while increasing proportions of D7$^+$ and D1/D7$^+$ populations (Fig. 4). These effects were dose dependent, requiring a concentration of at least 10 ng/ml. Increasing concentration to 20 ng/ml failed to increase the influence of cytokines on monocyte differentiation with the exception of the effect of IL-4 on D1$^+$ populations which were further raised by the use of this concentration. Direct comparison of Th1 and Th2-derived cytokines at optimal doses revealed that IL-2 had no effect on differentiation (Fig. 5), while both IFN-γ (Th1-derived) and Il-4 (Th2-derived) increased proportions of D1$^+$ cells in the maturing monocyte population (Figs 5 and 6); while IL-10 (Th2-derived) down-regulated this population and increased proportions of D7$^+$ and D1/D7$^+$ cells (Fig. 6). To further investigate the paradoxical effects of the Th2-derived cytokines, IL-4 and IL-10, experiments were performed whereby both these cytokines were added to the monocyte cultures simultaneously. The opposing effects of IL-4 and IL-10 on D1 and D7 expression cancel out when the two cytokines are used in combination (Fig. 6).

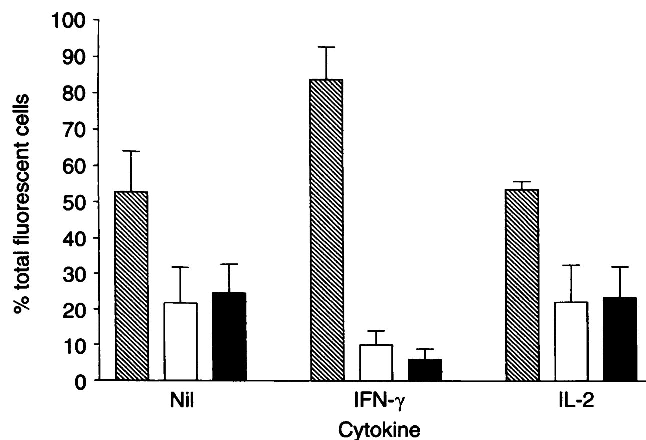

Figure 5. The relative proportions of D1$^+$ cells (▨); D7$^+$ cells (□) and D1/D7$^+$ cells (■) derived from monocytes after 7 days culture in median alone (nil) or with 2 days contact with Th1-derived cytokines IFN-γ and IL-2. Mean and standard error given from nine experiments. Using ANOVA $P = 0.003$ for D1, $P = 0.02$ for D7 and $P = 0.02$ for D1/D7.

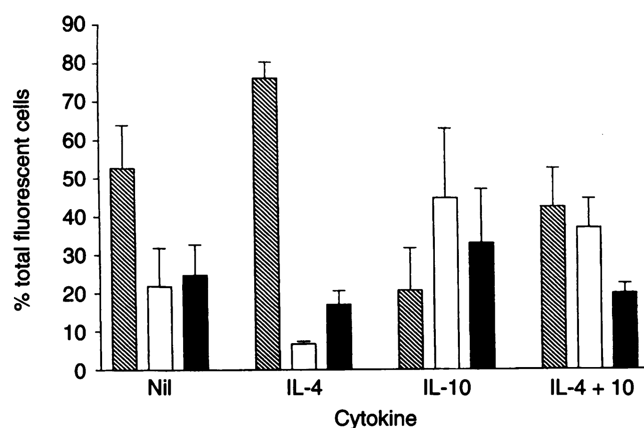

Figure 6. The relative proportions of D1$^+$ (▨); D7$^+$ (□) and D1/D7$^+$ (■) macrophages derived from monocytes after 7 days culture in median alone (nil) or with 2 days contact with IL-4, IL-10 or a combination of both. Mean and standard error given from nine experiments. Using ANOVA $P<0{\cdot}0001$ for D1, $P=0{\cdot}004$ for D7 and $P=0{\cdot}004$ for D1/D7.

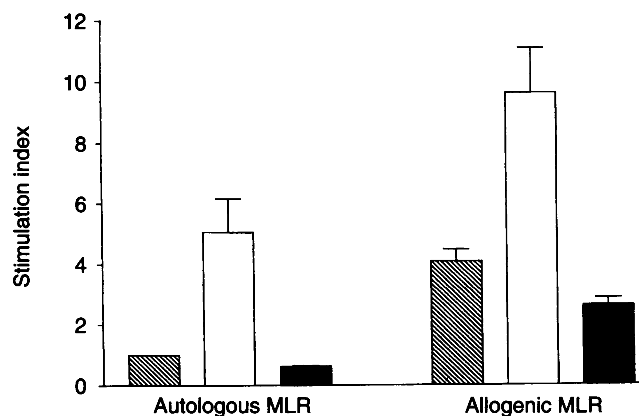

Figure 7. Autologous and allogeneic stimulation of peripheral blood mononuclear cells by 7 day cultures of monocytes without addition (▨); or following addition of IFN-γ (□); or IL-10 (■) to the cultures on day 5. Experiment performed three times, bars represent mean and standard error of stimulation index. (Counts per minute for autologous reactivity without cytokine addition were reduced to unity and all other results are represented as a stimulation index in relation to this result). $P<0{\cdot}001$ by ANOVA.

Cytokine contact affects HLA-DR expression

Expression of HLA-DR was measured in the whole macrophage pool after the peripheral blood monocytes had been cultured for 7 days. Cells cultured in the presence of IFN-γ or IL-4 showed significantly raised expression of HLA-DR in both cases, while IL-10 addition significantly reduced HLA-DR expression ($P<0{\cdot}01$ by ANOVA) (data not shown). IL-2 had no effect on HLA-DR expression. Overall however, these results revealed that membrane antigens known to be directly relevant to cell function may be modulated by contact with T-cell cytokines.

Modulation of monocyte differentiation may have functional significance

Monocytes cultured in the presence of IFN-γ or IL-10 for 48 hr were harvested at day 7 and admixed with autologous or allogeneic peripheral blood mononuclear cells (PBMCs). Cells treated with IFN-γ promoted a five-fold increase in autologous MLR stimulation index, while those treated with IL-10 reduced autologous reactivity by over 50% (Fig. 7). A similar effect of cytokine contact was recorded when allogeneic MLR was studied. 7 day cultures of monocytes untreated with cytokines produced a stimulation index of 3·9 when add-mixed with allogeneic PBMCs. This reactivity was increased to a stimulation index of 9·6 when IFN-γ-treated monocytes were used as the stimulatory population; and reduced to a stimulation index of 2·4 when differentiating monocytes treated with IL-10 were used as the stimulatory population. ($P<0{\cdot}0001$ using two-way ANOVA).

Thus cytokine contact promoting increased proportions of D1$^+$ cells in culture (IFN-γ treatment, see above) significantly increased the T-cell stimulatory capacity of the macrophage pool. Conversely, treatment increasing the D1/D7$^+$ population (contact with IL-10) in culture resulted in suppression of T-cell stimulation when subsequently investigated in autologous or allogeneic MLR (Fig. 7).

DISCUSSION

The differentiation of monocytes into macrophages is associated with changes in cell phenotype identified by mAbs RFD1 and RFD7.[3,4] The current study confirms that on maturation, monocytes (that do not normally express the antigens seen by these monoclonal antibodies), develop into three subsets of mature cells identified as D1$^+$, D7$^+$, and D1/D7$^+$ 0. These subsets appear morphologically similar but have been shown by this laboratory and many others (see introduction) to exhibit differences in surface receptor expression, enzyme content, and function.[3,9] For example, D1$^+$ cells act as inducers of T-cell stimulation, while D1/D7$^+$ cells suppress T-cell responsiveness.[3] Furthermore, previous studies in this laboratory using D1 and D7 as blocking antibodies demonstrated a direct association between the functional changes in an MLR and the expression of D1 and D7 antigens on macrophages.[10] Thus, within a pool of mature macrophages overall functional capacity may be determined by the balance created by the relative proportions of these subsets.

It is well established that the functional capacity of mature macrophages can be influenced by cytokines such as IFN-γ released from T cells.[30] Previous studies from this laboratory have demonstrated that IFN-γ also influences the maturation of monocytes.[31] The present study goes further in demonstrating that monocyte maturation can also be influenced by IL-4 and Il–10. It is accepted that other soluble T-cell products not tested here may also exert effects on this differentiation.

Table 1. Effect of T-cell cytokines on macrophage subpopulations

	Macrophage phenotype		
	D1+	D7+	D1+D7+
IL-4	↑	↓	↓
IL-10	↓	↑	↑
IL-2	–	–	–
IFN-γ	↑	↓	↓

However, the lack of any detectable effect of IL-2 would suggest that this is not a non-specific phenomenon and is thus under some form of regulation.

The current data contain three fascinating observations. Firstly, a comparison of the effects of IL-4 and IFN-γ reveals that cytokines derived from both Th1-like T cells (IFN-γ) and Th2-like T cells (IL-4) can both promote the emergence of a macrophage pool with a dominant T-cell stimulatory capacity (by promoting the development of the $D1^+$ phenotype). Secondly, a comparison of the effects of IFN-γ and IL-10 (the latter promoting increased proportions of $D1/D7^+$ cells) reveals that Th1-derived and Th2-derived factors can have divergent effects on monocyte maturation. Thirdly, the contrasting effects of IL-4 and IL-10 (both Th2-derived cytokines) emphasises the potential complexity of T-cell regulation of macrophage maturation.

Reviews of cytokine mediation of T cell/macrophage interaction[32] do not necessarily take into account the possibility of differential effects on specific macrophage sub-populations. This question has been addressed, with the conclusion that Th1 and Th2 cytokines may act on different macrophage populations.[33] The present study would support this, and go further in indicating that the overall functional capacity of a mature macrophage pool may be regulated by T-cell control of the relative proportions of functionally distinct sub-populations developing from monocytes. Previous observations that IFN-γ up-regulates antigen presenting capacity[18] and MHC class II expression[34] while IL-10 blocks tumour necrosis factor-α (TNF-α) production[35,36] reduces microbicidal activity[17,37] and suppresses antigen presentation[38] can all be explained on the basis of changes in the local development of dominance of one macrophage subset over another. Reports that cytokine-induced changes are greater on cultured rather than freshly isolated monocytes[39] is consistent with the observations made here, that day 5 addition of cytokine was most effective. This could be taken to indicate that changes to macrophage phenotype and possibly function may be induced in relatively mature cells.

Such a possibility could have major importance in understanding the pathogenesis of chronic inflammatory diseases where populations of macrophages constitute a significant component of the cellular infiltrates involved. For example, this laboratory has previously reported that the immunopathology in inflammatory bowel disease[13] and in bronchial asthma[16] are characterized by a gross imbalance in the relative proportions of phenotypically defined inductive and suppressive macrophages. This may be of particular relevance in the latter situation as local populations of suppressive macrophages have been seen to be an important regulator of T-cell activation at this site.[39] Such control is mediated by down-regulation of antigen-presenting cell function.[40] The results of MLR assays reported in this paper offer evidence to support the suggestion that IL-10 and IFN-γ contact with monocytes can promote suppression or enhancement of T-cell stimulation, respectively. It could be suggested that such mechanisms could form the basis of cell-mediated immune regulation *in situ*. It might be concluded, therefore, that control of the balance of functionally distinct macrophage subsets represents a major role of T-cell derived cytokines. The fact that macrophages themselves produce cytokines such as IL-1, TNF-α, transforming growth factor-β (TGF-β) and indeed IL-10 all of which are known to influence T-cell subset proportions and function[41–44] would indicate that mutual regulation may be exerted between T cells and macrophages. It is tempting to speculate that cytokine driven two-way signalling between T cells and macrophages is designed to sustain the appropriate balance of subsets of each cell type in any given environment. The present data is germaine to this argument as it reveals a plasticity of macrophage phenotype and function under T-cell control, which in turn may regulate the reactive potential of the T-cell populations.

ACKNOWLEDGMENTS

The authors thank Ms Sharon Bernard for her expert technical assistance. Vincent Tormey and Colm Leonard are in receipt of Research Fellowships from the British Council in Ireland. This work was supported by Glaxo Research and Development UK Ltd.

REFERENCES

1. Toews G.B., Vial W.C., Dun M.M. *et al.* (1984) The accessory cell function of human alveolar macrophages in specific T cell proliferation. *J Immunol* **132,** 181.
2. Thepen T., Kraal G. & Holt P.G. (1994) The role of alveolar macrophages in regulation of lung inflammation. *Annals NY Acad Sci* **725,** 200.
3. Spiteri M.A. & Poulter L.W. (1991) Characterisation of immune inducer and suppressor macrophages from the normal human lung. *Clin Exp Immunol* **83,** 157.
4. Poulter L.W., Campbell D.A., Munro C. & Janossy G. (1986) Discrimination of human macrophages and dendritic cells by means of monoclonal antibodies. *Scand J Immunol* **24,** 351.
5. Lenz A., Heine M., Schuler G. & Romani P. (1993) Human and murine dermis contain dendritic cells. Isolation by means of a novel method and phenotypical and functional characterisation. *J Clin Invest* **92,** 2587.
6. Seldenrijk C.A., Drexhage H.A., Meuwissen S.G., Pale S.T. & Meijer C.J. (1989) Dendritic cells and scavenger macrophages in chronic inflammatory bowel disease. *Gut* **30,** 486.
7. Teunissen M.B., Wormmeester J., Krieg S.R. *et al.* (1990) Human epidermal langerhans cells undergo profound morphologic and phenotypical changes during *in vitro* culture. *J Immun Dermatol* **94,** 166.
8. Zheng H., Teschler H., Guzman J., Hubne K., Striz J., & Costabel U. (1995) Alveolar macrophages TNFα release and BAL cell phenotype in sarcoidosis. *Am Rev Respir Crit Care Med* **153,** 1061.
9. Spiteri M.A., Clark S.W. & Poulter L.W. (1992) Isolation of phenotypically and functionally distinct macrophage subpopulations from human bronchoalveolar lavage. *Eur Resp J* **5,** 717.
10. Poulter L.W. & Burke C.M. (1996) Macrophages and allergic lung disease. *Immunobiology* **195,** 574.
11. Hutter C. & Poulter L.W. (1992) The balance of macrophage subsets may be customised at mucosal surfaces. *FEMS Microbiol Immunol* **105,** 309.
12. Poulter L.W. (1991) Changes in lung macrophages during disease. *FEMS Micro Immunol* **64,** 327.
13. Allison M.C. & Poulter L.W. (1991) Changes in phenotypically distinct mucosa macrophage populations may be a prerequisite for the development of inflammatory bowel disease. *Clin Exp Immunol* **85,** 504.
14. Spiteri M.A., Clarke S.W. & Poulter L.W. (1992) Alveolar macrophages that suppress T cell responses may be crucial to the pathogenetic outcome of pulmonary sarcoidosis. *Eur Resp J* **5,** 394.
15. Lim S.G., Condez A. & Poulter L.W (1993) Mucosal macro-

phages of the gut in HIV: decrease in antigen presenting cell phenotype. *Clin Exp Immunol* **92,** 442.

16. POULTER L.W., JANOSSY G., POWER C., SREENAN S. & BURKE C. (1994) Immunological/physiological relationships in asthma: potential regulation by lung macrophages. *Immunol Today* **15,** 258.
17. CAMPBELL P.A. (1994) Macrophage–*Listeria* interactions. *Immunol Series* **60,** 313.
18. YOUNG H.A. & HARDY K.J. (1995) Role of IFNγ in immune cell regulation. *J Leuk Biol* **58,** 373.
19. DE WAAL MALEFYT R., ABRAMS J., BENNETT B., FIGDOR C.G. & DE VRIES J.E. (1991) Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med* **174,** 1209.
20. FIORENTINO D.F., ZLOTNIK A., MOSMANN T.R., HOWARD M. & O'GARRA A. (1991) IL-10 inhibits cytokine production by activated macrophages. *J Immunol* **147,** 3815.
21. FLORES VILLANUEVA P.O. & REISER, H. (1994) Regulation of T helper cell responses in experimental murine schistosomiasis by IL-10. Effect of expression of B7 and B72 costimulatory molecules by macrophages. *J Immunol* **153,** 5190.
22. DING L., LINSLEY P.S., HUANG L.Y., GERMAIN R.N. & SHEVACH E.M. (1993) IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the upregulation of B7 expression. *J Immunol* **151,** 1224.
23. RUPPERT J., FRIEDRICK D., XU H. & PETERS J.H. (1991) IL-4 decreases the expression of the monocyte differentiation marker CD14 paralleled by an increasing accessory potency. *Immunobiology* **182,** 449.
24. LEE J.D., RHODES K. & ECONOMOU J.S. (1995) IL-4 inhibits the expression of tumor necrosis factors α and β, interleukins 1b and 6 and interferon γ. *Immunol & Cell Biol* **73,** 57.
25. HOGG N., MACDONALD S., SHISARENKO M., BEVERLEY L. (1984) Monoclonal antibodies specific for human monocytes, granulocytes and endothelium. *Immunology* **53,** 753.
26. KELLY P.M.A., BLISS E., MORTON J.A., BURNS J. & MAGEE J.O'D. (1988) Monoclonal antibody EBM11 in cellular specificity for human macrophages. *J Clin Pathol* **41,** 510.
27. JANOSSY G., BOFILL M. & POULTER L.W. (1986) Two colour immunofluoresence analysis of the lymphoid system with monoclonal antibodies. In: *Immunocytochemistry Today* (eds J. Polak & S. Van Noorden), p. 438. J. Wright & Sons, Bristol.
28. CAMPBELL D.A., DUBOIS R.M., BUTCHER R.G. & POULTER L.W. (1986) The density of HLA-DR antigen expression is increased in pulmonary sarcoidosis. *Clin Exp Immunol* **65,** 165.
29. POULTER L.W., POWER C. & BURKE C. (1990) The relationship between bronchial immunopathology and hyperresponsiveness in asthma. *Eur Resp J* **3,** 792.
30. DOHERTY T.M. (1995) T cell regulation of macrophage function. *Curr Opin Immunol* **7,** 400.
31. POULTER L.W., ROOK G.A.W., STEELE J. & CONDEZ A. (1987) Influence of 1-25 $(OH)_2$ vitamin D3 and gamma interferon on the phenotype of human peripheral blood monocyte-derived macrophages. *Infect Immun* **55,** *2017*
32. BELARDELLI F. (1995) Role of interferons and other cytokines in the regulation of the immune response. *APMIS* **103,** 161.
33. MOLINA I.J. & HUBER B.T. (1991) Regulation of macrophage activation markers by IL-4 and IFNγ is sub-population specific. *Cell Immunol* **134,** 241.
34. TRINCHIERI G. & PERUSSIA B. (1985) Immune interferon: a pleitrophic lymphokine with multiple effects. *Immunol Today* **6,** 131.
35. WEISMANN D., POLI G. & FAUCI A.S. (1994) Interleukin-10 blocks HIV replication in macrophages by inhibiting the autocrine loop tumour necrosis factor alpha and interleukin 6 induction of virus. *AIDS Res & Human Retroviruses* **10,** 1199.
36. HART P.H., AHEN M.J., SMITH M.D. & FINLAY-JONES J.J. (1995) Comparison of the suppressive effects of interleukin-10 and interleukin-4 on synovial fluid macrophages and blood monocytes from patients with inflammatory arthritis. *Immunology* **84,** 536.
37. FERNANDES D.M. & BALDWIN C.L. (1995) Interleukin downregulates protective immunity to *Brucella abortus*. *Infect Immun* **63,** 1130.
38. MACATONIA S.E., DOHERTY T.M., KNIGHT S.C. & O'GARRA A. (1993) Differential effect of IL-10 on dendritic cell-induced T cell proliferation and IFNγ production. *J Immunol* **150,** 3755.
39. HOLT P.G., MCMENAMIN C., SCHON-HEGRAD M.A. *et al.* (1991) Immunoregulation of asthma: control of T lymphocyte activation in the respiratory tract. *Eur Resp J* **4,** (Suppl. 13), 6 s.
40. HOLT P.G., OLIVER J., BILYK N., MCMENAMIN C., MCMENAMIN P.G., KRAAL G. & THEPEN T. (1993) Downregulation of antigen presenting cell function (s) of pulmonary dendritic cells *in vivo* by resident alveolar macrophages. *J Exp Med.* **177,** 397.
41. SKEEN M.J. & ZIEGLER H.K. (1995) Activation of gamma delta T cells for production of IFN-gamma is mediated by bacteria via macrophage derived cytokines IL-1 and Il-1 *Immunol* **154,** 5832.
42. ZUNNIGA-PFLUCKER J.C. & DI J. LENARDO M.J. (1995) Requirment for TNFα and IL-1α in fetal thymocyte commitment and differentiation. *Science* **268** (1906)
43. SWAIN S.L., HUSTON G., TONKONOGY S. & WEINBERG A. (1991) Transforming growth factor and IL-4 cause helper T cell precursors to develop into distinct effector helper cells that differ in lymphokine secretion pattern and cell surface phenotype. *J Immunol* **147,** 299.
44. MOSMANN T.R. (1991) Regulation of immune responses by T cells with different cytokine secretion phenotype: role of new cytokine, cytokine synthesis inhibiting factor (IL-10). *Int Arch All Appl Immunol* **94,** 110.