MODULATION OF PHENOTYPIC AND FUNCTIONAL PROPERTIES OF HUMAN PERIPHERAL BLOOD MONOCYTES BY IL-4

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Highly purified peripheral blood monocytes were cultured in the presence of rIL-4. Major changes in the morphology of the monocytes were observed. After day 5 of culturing the cells acquired a macrophage-like appearance, with increased cell size and extensive processes, suggesting that IL-4 may induce monocyte-macrophage differentiation. This notion is supported by the observed increased expression of MHC class II Ag, which is thought to be associated with monocyte differentiation. Exposure of monocytes to IL-4 resulted in a dose-dependent increase of the expression of MHC class II Ag, which became apparent after only 20 h of incubation. Maximal expression was obtained after incubation for 6 days, and persisted throughout the whole culture period. Similarly, IL-4 increased the expression of R for C3bi and p150.95 Ag, two members of the leukocyte function-associated Ag 1 family, whereas the expression of the third member, leukocyte function-associated Ag 1, remained unchanged during culture. Furthermore, it was shown that IL-4 inhibited the secretion of cytostatic and chemotactic compounds. Supernatants of monocytes cultured with IL-4 were, in contrast to control cultures, much less effective in inhibiting the growth of A375 melanoma cells. In addition, these supernatants failed to direct the migration of freshly isolated monocytes in a chemotaxis assay. Further analysis revealed that these supernatants exhibited reduced IL-1 activity, as measured in a mouse thymocyte proliferation assay, which might explain the low cytostatic and chemotactic activity.

Taken together these results show that IL-4 modulates monocyte phenotype and function and may induce monocyte-macrophage differentiation in vitro.

Since IL-4 was first described as BSF-1, this IL has been shown to have important pleiotropic biologic effects in the mouse as well as in the human system. Murine BSF-1 (IL-4) activates preactivated B cells to proliferate (1), and presumably resting B cells to enter the S phase in response to appropriate stimuli (2, 3). In addition, mouse IL-4 has been shown to induce/enhance the expression of class II MHC Ag on B lymphocytes (4, 5) and to stimulate the growth of mast cells (6) and activated T cells (6–8).

By using RNA from Con A-activated human T cells, a cDNA coding for the human homologue of mouse IL-4 was isolated. The human rIL-4, synthesized with this cDNA in cos 7 cells (9), stimulated the growth of preactivated B and T cells (9, 10) and induces MHC class II Ag expression on normal resting B cells (T. Defrance, unpublished data) and malignant B cells7 comparable with mouse IL-4.

It is well established that quantitative variation in class II MHC Ag plays a major role in the regulation of Ig (11, 12). Therefore, IL-4 may enhance the Ig by its positive regulation of class II MHC Ag on B cells. Not only B cells but also monocytes and macrophages show a variation of MHC class II expression, which is closely related to their function in the Ig, and can be modulated by T cell-derived lymphokines (13).

In the present study we investigated the effect of human rIL-4 on the modulation of MHC class II Ag expression on human peripheral blood monocytes. The monocytes were isolated in a way that prevented activation of the cells, so that the cells expressed relatively low levels of class II MHC Ag. Here it is shown that IL-4 enhances the expression of class II MHC Ag on human monocytes. Furthermore, it is reported that IL-4 induces a selective up-regulation of the expression of CR3 and p150.95 adhesion associated Ag, a macrophage-like morphology and a reduction of the capacity to secrete IL-1 like activity. The possibility is discussed that IL-4 may induce monocyte-macrophage differentiation in vitro.

MATERIALS AND METHODS

Isolation of monocytes. Human peripheral blood monocytes were isolated from 500 ml of blood from normal human donors as described previously (14, 15). First, mononuclear cells were separated by density centrifugation with a blood component separator. Subsequently the mononuclear cells were fractionated into lymphocytes and monocytes by centrifugal elutriation. The monocyte preparation was over 95% pure as judged by nonspecific esterase staining and contained more than 98% viable cells. Contamination with lymphocytes and granulocytes was generally less than 2 and 3%, respectively.

Monocyte cultures. Monocytes were cultured in a modified Iscove’s medium (16). Monocytes were incubated in medium supplemented with 2% autologous heat-inactivated serum. The culture medium was found to be endotoxin-free (defined as less than 0.2 ng/ml of endotoxin as quantified by the Limulus amebocyte lysate assay). Monocytes (4 × 10⁶/ml) were incubated
at 37°C, 5% CO₂ and 100% humidity in Teflon bags (Jansen’s MNL, St. Niklaas, Belgium). Monocytes cultured for 1 to 14 days were collected from the Teflon bags. After removal of the culture supernatant the cells were washed and resuspended in PBS containing 0.5% BSA and 0.02% azide and kept on ice until used as source of IL-4. As a control medium, supernatant from mock-transfected cos 7 cells, processed in the same way, was used (mock-medium). In some experiments purified IL-4 (a gift from Dr. S. Nagabhushan, Schering Corp., Bloomfield, NJ) was used. Purified IL-4 or culture supernatant containing IL-4 was used in the various assays yielding similar results.

Detection of surface membrane determinants. Class II MHC and LFA-1 family Ag expression was measured on freshly isolated and cultured monocytes. The following anti-class II MHC antibodies were used. QS/13 (lgG2a) which detects a determinant common to HLA-DR and HLA-DP molecules (17) was a gift from Dr. S. Ferrone, Medical College, Vahalla, NY, and SPV-L3 (lgG1), which reacts with a monomorphic determinant on HLA-DQ molecules (18). The anti-LFA-1 family antibodies used were: SPV-L7 (lgG1) which recognizes the α-chain of LFA-1 (19), Bear-1 (lgG1) which is similar to anti-CR3 (20) and detects the α-chain of CR3, S HCl-3 (lgG2b), kindly provided by R. Schwarting, Freie Universitat Berlin, Berlin, West Germany (21), identifies the α-chain of CR4 (22, 23). Other reagents were used in the various assays yielded similar results.

Effect of IL-4 on the expression of HLA-DQ Ag by human monocytes. Monocytes cultured in the presence of IL-4 show an increase in the expression of HLA-DR and HLA-DQ Ag as compared with control cultures. Culturing of monocytes in medium results in an enhanced HLA class II Ag expression, but in spite of this IL-4 causes a further increase. Maximal increase in expression was found at concentrations of 50 U of purified IL-4 (Table I). This concentration was used for further phenotypic and functional characterization. As a control no increase in expression of a 135-kDa monocyte-specific Ag was observed after culturing with IL-4 compared with control cultures. The enhanced expression of class II MHC Ag was already observed after 1 day of culturing with and without IL-4, and maximal induction was found at day 6 (Table II). Compared with control cultures a 2- to 6-fold increase in HLA-DR expression was present on monocytes cultured with IL-4, which persisted for at least 14 days. Similar results were found with regard to the expression of HLA-DQ (Table II).

Effect of IL-4 on the expression of LFA-1 family Ag by human monocytes. All three Ag of the LFA-1 family (LFA-1, Mac-1, CR3) are increased in expression on monocytes cultured with IL-4. The effects are dose dependent (Table II). Compared with control cultures a 2- to 6-fold increase in HLA-DR expression was present on monocytes cultured with IL-4, which persisted for at least 14 days. Similar results were found with regard to the expression of HLA-DQ (Table II).
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1, CR3, and p150.95) are expressed on human monocytes (26). Because these Ag function in monocyte-adhesion reactions, which are important in host defenses (26), we investigated the effect of IL-4 on the expression of LFA-1 family Ag on human monocytes. As is shown in Table III incubation of monocytes with IL-4 for 3 days modulates the expression of CR3, the p150.95 Ag and the common LFA-1 family β-chain, whereas the expression of LFA-1 α was not affected. Again, no change in the level of expression of a 135-kDa monocyte-specific Ag was observed upon culture with IL-4 (Table III). The up-regulation of CR3 and p150.95 becomes apparent within 20 h of incubation and the Ag remain elevated throughout the whole culture period (7 days, results not shown).

Effect of IL-4 on the morphology and metabolic activity of cultured human monocytes. The morphology of monocytes cultured with IL-4 does not differ from those cultured in the absence of IL-4 during the first 5 days of culturing. After this period the monocytes cultured in the presence of IL-4 show a strikingly different morphology compared with control cultures. At day 6 the cells acquire a macrophage-like, dendritic cell morphology and are considerably increased in size compared with control cultures. In addition they have extensive processes (Fig. 1). These results were confirmed by the increased forward light scatter signal (which is a measure for cell size) obtained with flow cytometry of monocytes cultured in the presence of IL-4 compared with control cultures (Table IV). The increase in cell size was accompanied by an increase in protein synthesis measured by [35S]methionine incorporation into cultured monocytes (Table IV). Furthermore, the phagocytic activity of latex beads of monocytes after culture either with or without IL-4 was not impaired (data not shown). These data suggest that IL-4 induces maturation or differentiation of human monocytes.

Effect of IL-4 on human monocyte migration and chemotaxis. Monocytes cultured for 20 h in the presence or absence of IL-4 were tested on their capacity to produce chemotactic cytokines. After this culture period the chemotactic activity of the supernatant was tested on freshly isolated monocytes and compared with control cultures. At day 6 the cells acquire a macrophage-like, dendritic cell morphology and are considerably increased in size compared with control cultures. In addition they have extensive processes (Fig. 1). These results were confirmed by the increased forward light scatter signal (which is a measure for cell size) obtained with flow cytometry of monocytes cultured in the presence of IL-4 compared with control cultures (Table IV). The increase in cell size was accompanied by an increase in protein synthesis measured by [35S]methionine incorporation into cultured monocytes (Table IV). Furthermore, the phagocytic activity of latex beads of monocytes after culture either with or without IL-4 was not impaired (data not shown). These data suggest that IL-4 induces maturation or differentiation of human monocytes.

Effect of IL-4 on human monocyte migration and chemotaxis. Monocytes cultured for 20 h in the presence or absence of IL-4 were tested on their capacity to produce chemotactic cytokines. After this culture period the chemotactic activity of the supernatant was tested on freshly isolated monocytes and compared with the chemotactic activity of control medium with and without IL-4. Figure 2 shows that monocytes cultured in medium or mock-medium produce a factor that is chemotactic for freshly isolated monocytes in a concentration-dependent fashion. Maximal chemotactic activity was obtained at an 1/10 dilution of the monocyte supernatant. The percentage of chemotaxis was comparable to the chemotactic responses obtained if fMLP or rIL-1 were used as chemoattractant (28 ± 3 and 21 ± 4, respectively). Addition of IL-4 during the chemotaxis assay did not affect the migratory response. However, supernatants of monocytes cultured in the presence of IL-4 did not contain chemotactic activity. In addition, we investigated the chemotactic response of monocytes which were cultured for 20 h. Although supernatants of monocytes cultured in the presence of IL-4 did not contain chemotactic activity, the monocytes themselves were not affected in their capacity to respond to fMLP (Fig. 3). Together, these results indicate that IL-4 inhibits the secretion of chemotactic factors by human monocytes, whereas it does not affect the ability of these cells to respond to fMLP.

Effect of IL-4 on the secretion of cytostatic factors by human monocytes. Supernatants of activated human peripheral blood monocytes have been shown to contain cytokines with growth inhibitory activity for the human melanoma cell line A375 (27–30). This cell line was used to study the effects of IL-4 on the production of cytostatic factors by cultured monocytes. Supernatants of monocytes cultured for 20 h in the presence or absence of IL-4 were added to A375 melanoma cells. As shown in Figure 4 the supernatants of monocytes cultured in the absence of IL-4 strongly reduced the number of A375 colonies, whereas supernatants of monocytes cultured with IL-4 apparently contained less cytostatic factors. The reduction of the growth inhibitory activity is already evident at 48 h of culture (Fig. 5). Addition of IL-4 directly to the A375 cultures did not affect colony formation. From these data it can be concluded that monocytes cultured in the presence of IL-4 have a reduced capacity to secrete cytostatic factors.

Effect of IL-4 on the synthesis of IL-1 by human monocytes. It has been shown that human monocytes secrete IL-1 upon culture (31). In addition IL-1 can act as a chemotactic factor for human monocytes (32) and has cytostatic/cytotoxic effects on certain human tumor cell lines (27). In order to investigate whether the reduced chemotactic and cytostatic activities described above are attributed to a reduction in their capacity to produce IL-1 activity, supernatants of monocytes cultured with and without IL-4 were tested for IL-1 activity. IL-1 activity was measured by [3H]thymidine incorporation in murine thymocytes stimulated with Con A. Supernatants of monocytes cultured in the presence of IL-4 show a strong reduction of the proliferation of thymocytes (Fig. 6) which might explain the reduced chemotactic and cytostatic activity found in supernatants from monocytes cultured in the presence of IL-4. The reduced IL-1 activity could not be attributed to IL-4 still present in the supernatant, because the thymocyte proliferation in control medium containing IL-4 was not inhibited compared with medium without IL-4.

**TABLE III**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Ag Recognized</th>
<th>Cultured for 3 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+ IL-4</td>
</tr>
<tr>
<td>IgG1 control</td>
<td>IgG (ID)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>IgG2b control</td>
<td>IgG (ID)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>1/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFW-L7</td>
<td>LFA-1 (α-chain)</td>
<td>7 (88)</td>
</tr>
<tr>
<td>Bear 1</td>
<td>CR3 (α-chain)</td>
<td>10 (92)</td>
</tr>
<tr>
<td>S HCl-S</td>
<td>p150.95 (α-chain)</td>
<td>23 (87)</td>
</tr>
<tr>
<td>CLB 54</td>
<td>LFA-1, CR3, p150.95 (common β-chain)</td>
<td>11 (92)</td>
</tr>
<tr>
<td>Sam 1</td>
<td>135-kDa monocyte Ag</td>
<td>5 (99)</td>
</tr>
</tbody>
</table>

a Monocytes were labeled with mAb and GAM F(ab')2 FITC after 3 days of culture with or without 50 U of IL-4/ml. Data are expressed as rFI and percentage positive cells (in parentheses). For each value more than 10,000 cells were counted. A representative experiment out of three is shown.

**DISCUSSION**

The recent availability of human rIL-4 gave us the opportunity to assess the action of this cytokine on human monocytes. By advanced blood component separation in combination with centrifugal elutriation it was possible to isolate large amounts of highly purified monocytes that are equal in size and free of attached thrombocytes (14, 15). Furthermore it has been shown that monocytes isolated according to this procedure, are not activated by the iso-
Figure 1. Effect of IL-4 on the morphology of human monocytes. Monocytes were cultured for 7 days with mock-medium (a) or with 50 U IL-4/ml (b). Morphologic changes became apparent on day 5. No significant shape changes were observed during further incubation (14 days) (magnification × 200).
TABLE IV

<table>
<thead>
<tr>
<th>IL-4a Added</th>
<th>Expt. 1</th>
<th>Expt. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell sizeb</td>
<td>ch. 83</td>
<td>ch. 85</td>
</tr>
<tr>
<td>+</td>
<td>ch. 112</td>
<td>ch. 117</td>
</tr>
<tr>
<td>Protein synthesisc</td>
<td>500 ± 42</td>
<td>750 ± 53</td>
</tr>
<tr>
<td>[35S]methionine incorporation (cpm × 10^-3)</td>
<td>+ 800 ± 64</td>
<td>1125 ± 62</td>
</tr>
</tbody>
</table>

a Monocytes were cultured with or without 50 U IL-4/ml for 6 days.
b Cell size is expressed as mean channel (ch.) number of the forward light scatter signal measured by flow cytometry.
c [35S]methionine incorporation was determined as indicated in Materials and Methods. Data are presented as mean ± SD of sixfold values.

Figure 2. Effect of IL-4 on the production of chemotactic factors by human monocytes. Directed migration response of freshly isolated monocytes to diluted supernatant of monocytes (mo-sup) cultured for 20 h with medium (white triangle), mock-medium (gray square) or 50 U IL-4/ml (black square). Data are expressed as migration index:

Migration response to mo-sup

Migration response to control medium

Data of one representative experiment out of four are shown. The migration responses to control medium with or without IL-4 were 12 ±3% and 11 ±2%, respectively. SD of triplicate values did not exceed 5%.

Figure 3. Migratory response of cultured monocytes incubated for 20 h in medium, mock medium, or 50 U IL-4/ml. The lower compartment of the chemotaxis chamber was filled with medium (gray bars) or medium with 10^-8 M fMLP (open bars). Values represent mean ± SD of one representative experiment out of five.

Figure 4. Effect of IL-4 on the secretion of cytostatic factors by human monocytes. Colony formation of A375 cells was determined in the presence of supernatant of monocytes (mo-sup) cultured for 20 h in medium (white triangle), mock-medium (gray square) or 50 U IL-4/ml (black square). Data are expressed as percentage inhibition of colony formation of A375 cells according to the formula:

\[ \text{inhibition} = \frac{\text{number of colonies in mo-sup}}{\text{number of colonies in control medium}} \times 100\% \]

Data of one representative experiment out of four are shown. The number of colonies formed in control medium with or without IL-4 were 91 ±4 and 87 ±5, respectively. SD of the number of colonies of six wells did not exceed 8%.

Monocytes play a central role in the regulation of IgR and modulation of the expression of class II MHC Ag seems to be a closely correlated event (13, 33). Upon culture the expression of class II MHC Ag on monocytes increases rapidly (34) (Table II). In addition culture supernatants of lectin-activated lymphocytes can increase the expression of these Ag and IFN-γ has been shown to be the inducing agent (35). Here it is demonstrated that IL-4 also increases the expression of class II MHC Ag (Table II). The up-regulation of class II MHC Ag on monocytes by IL-4 could not be attributed to other compounds in the cos 7 transfection supernatants, since purified rIL-4 has similar effects. Moreover, HLA-DR expression was not affected by supernatants of mock-transfected cos 7 cells. The observation that IL-4 modulates class II Ag expression in human peripheral blood monocytes may indicate that IL-4 has important immunoregulatory activities. An intriguing question is whether this increased MHC class II expression has any functional consequences, such as improved Ag-presenting capability of human monocytes. Zlotnik et al. (36) have already demonstrated that murine rIL-4 induced the Ag-presenting ability in mouse B cells or bone marrow-derived macrophages, and is accompanied with a small but significant increase in Ia Ag expression.

In addition to class II MHC Ag the LFA-1 family of molecules has been implicated in the function of monocytes. These Ag act as adhesion molecules mediating cell-substrate and cell-cell interactions of myeloid and lymphoid cells (26). When monocytes are cultured in the presence of IL-4 the expression of the CR3 and p150,95 Ag is up-regulated procedures. Such monocyte preparations therefore provide an excellent source to study whether IL-4 affects the phenotypic and functional characteristics of human monocytes.
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Figure 5. Effect of IL-4 on the secretion of cytostatic factors by human monocytes. Proliferation of A575 cells was measured in the presence of supernatant of monocytes (mo-sup) cultured in medium (Δ --- Δ), mock-medium (□ .......□), or 50 U IL-4/ml (■ — ■). Data are expressed as percentage inhibition of [³H]thymidine incorporation of A575 cells according to the formula:

\[
\frac{[³H]thymidine incorporation in mo-sup}{[³H]thymidine incorporation in control medium} \times 100\%.
\]

Data of two individual experiments are shown. The [³H]thymidine incorporation in control medium was 55,000 ± 4,900 and 21,000 ± 1,900, respectively. Similar values were obtained with control medium containing IL-4. SD of six samples did not exceed 10%.

![Graph showing inhibition of thymidine incorporation by IL-4](image)

Figure 6. Effect of IL-4 on the production of IL-1 by human monocytes. Monocytes were cultured for 20 h with (■ ----■) or without (Δ --- Δ) 50 U IL-4/ml. The IL-1 in the supernatant was determined by [³H]thymidine incorporation (cpm 10⁻³) in murine thymocytes activated by Con A. Values represent mean ±SD of one representative experiment out of three.

![Graph showing production of IL-1 by IL-4](image)

A similar selective up-regulation of CR3 and p150.95 Ag was found upon activation of monocytes by inflammatory stimuli (26). The surface expression of CR3 and p150.95 Ag is rapidly increased by mobilization of these Ag from intracellular pools to the cell surface within 30 min, but LFA-1 Ag expression was not increased upon activation (26). In addition, differentiation of myeloid cells results in a similar but much slower increase in the expression of CR3 and p150.95 Ag, whereas the LFA-1 Ag expression remains stable (37). These latter findings closely resemble the results obtained when monocytes are cultured in the presence of IL-4 (Table III).

Upon comparison of the data concerning enhanced expression of class II MHC molecules and LFA-1 family molecules with the literature (37, 38) it can be concluded that the phenotypic changes of monocytes cultured with IL-4 are similar to the changes observed when monocytes differentiate into macrophages, indicating that IL-4 induces differentiation of human monocytes. This notion is supported by the morphology seen after prolonged culture in the presence of IL-4 (Fig. 1).

Upon activation, monocytes secrete a large number of immunoregulatory factors which mediate multiple activities on a variety of cells (39). Here we demonstrate that IL-4 inhibits the secretion of such factors, especially those with autostimulatory chemotactic and cytostatic activity. However, since multiple monocyte products with similar biologic activity have been described, it is difficult to attribute the inhibitory activity of IL-4 to the reduced secretion of a single component. According to Nathan (39) many monocyte products are capable to act as chemotaxtractant. From these products IL-1 (32) and fibronectin fragments (40) have been shown to be chemotactic for human monocytes. Recently also other monocyte derived factors such as granulocyte-macrophage colony-stimulating factor (41) and TNF (42) were capable of inducing directed migration of human monocytes. With respect to the inhibition of cellular proliferation even more monocyte-derived products have been reported (39). Thus far, two candidates, IL-1 and TNF, inhibited both the growth of A375 cells and possess chemotactic activity (27–30, 32). We have started experiments to identify the factors secreted in reduced amounts after incubation of monocytes with IL-4. Figure 6 shows that IL-4 inhibits the production of IL-1-like activity identified by the reduced ability to co-stimulate (with Con A) the proliferation of mouse thymocytes (Fig. 6). Since TNF has not been reported to stimulate the thymocyte proliferation, these data may indicate that IL-4 acts predominantly on the secretion of IL-1, but its effect on the production of other monokines cannot be excluded.

Finally, loss of IL-1 activity is associated with the in vitro maturation of monocytes to macrophages (43) (see footnote 5). Also tissue macrophages failed to produce IL-1 (44, 45). These findings further support the hypothesis that IL-4 induces in vitro monocyte differentiation into macrophages.

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