REGULATION OF AMINOPEPTIDASE-N (CD13) AND FcεRIIb (CD23) EXPRESSION BY IL-4 DEPENDS ON THE STAGE OF MATURATION OF MONOCYTES/MACROPHAGES

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IL-4 has multiple biologic activities and it has been shown to have effects on B and T lymphocytes, mast cells, NK cells, and monocytes. We studied the influence of IL-4 on the expression of cell membrane determinants, in particular aminopeptidase-N (CD13) and FcεRIIb (CD23), on human peripheral blood monocytes. We compared the response of monocytes with the response of human alveolar macrophages and monocytic cell lines (U937 and THP1), as mature and more immature representatives of the mononuclear phagocyte system, respectively. A dose-dependent increase of the expression of CD13 Ag was observed when monocytes were cultured with IL-4. Kinetic analyses revealed that this induction was maximal after 2 to 3 days of culture and resembled the kinetics of IL-4-induced expression of FcεRIIb on monocytes. This IL-4-induced increase was absent when monocytes were cultured with IL-4 and an anti-IL-4 antiserum. Concomitantly, an IL-4-induced increase in leucine-aminopeptidase activity could be observed. Northern blot analysis showed that incubation of monocytes with IL-4 induced a marked increase in CD13 mRNA. Alveolar macrophages also exhibited an increase in CD13 Ag expression when exposed to IL-4. Surprisingly, IL-4 was unable to induce expression of FcεRIIb on alveolar macrophages. U937 and THP1 cells did not show an induction of CD13 Ag when cultured in the presence of IL-4. However, IL-4 did induce the expression of FcεRIIb on both cell lines, suggesting the presence of functional IL-4R. Our data demonstrate that IL-4 increases the expression of CD13 Ag on monocytes. This IL-4-induced increase can also be observed in more mature monocytic cells such as alveolar macrophages, but is absent in immature cells such as U937 or THP1 cells. This is functionally accompanied by an increase in leucine-aminopeptidase activity and may be part of the general activation of monocytes/macrophages by IL-4. In conclusion, the data suggest that IL-4 responsiveness, in particular the induction of CD13 Ag and FcεRIIb expression, may be dependent on the stage of maturation of monocytes/macrophages.

Initially, IL-4 was described to act as a differentiation factor for resting B lymphocytes (1). Nowadays, IL-4 has been shown to have a variety of effects on different cell types. It may act as a growth factor for activated T lymphocytes, thymocytes, NK cells (2-4), and mast cells (4, 5). It may modulate the expression of cell membrane determinants, as FcεRIIb (CD23) and class II MHC Ag on B lymphocytes (6, 7) and monocytes (8, 9), and IL-2R (CD25) on B lymphocytes (10). Furthermore, it has been shown that IL-4 is able to stimulate monocyte maturation accompanied by modulation of maturation markers as CD14 and RFD9. In the course of studying the influence of IL-4 on monocytes/macrophages we observed that IL-4 is also able to regulate the expression of another myeloid Ag, CD13 Ag.

Originally, the cell membrane glycoprotein (gp150) defined by CD13 mAb was seen as a marker for subpopulations of hemopoietic cells (11-14). Currently, it is known to be present on many nonhemopoietic tissues, including fibroblasts, the intestinal and renal tubular epithelium (15, 16), and on synaptic membranes of the central nervous system (17). As the glycoprotein has been identified as aminopeptidase-N (EC 3.4.11.2) (14, 18), it has been suggested that this cell membrane enzyme may play a role in modulating the activity of bioactive oligopeptides (19). In the knowledge that the effects of cytokines on a certain cell type may depend on the maturation or differentiation stage of the cell (20, 21), we studied the influence of IL-4 on the expression of the CD13 Ag on PBM as well as AM and monocytic cell lines (U937 and THP1), which can be regarded as more mature and immature representatives of the mononuclear phagocyte system, respectively. In this report, we demonstrate that IL-4 is able to induce a strong increase in expression of the CD13 Ag. This induction can be demonstrated on PBM and AM, but not on U937 and THP1 cells.

Received for publication November 13, 1991.
Accepted for publication June 2, 1992.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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4 Abbreviations used in this paper: PBM, peripheral blood monocytes; AM, alveolar macrophages; LAP, l-leucine-aminopeptidase; BAL, bronchoalveolar lavage; PCR, polymerase chain reaction; GAM, goat anti-mouse Ig antiserum.
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MATERIALS AND METHODS

Isolation of PBM. Human PBM were isolated from 500 ml blood from healthy volunteers as described elsewhere (22). Briefly, mononuclear cells were separated by density centrifugation with a component separator. Next, the mononuclear cells were fractionated into lymphocytes and monocytes by centrifugation elutriation. The monocyte preparation was over 95% pure and contained more than 98% viable cells as judged by trypan blue exclusion. Lymphocytes and granulocytes were not recovered in the monocyte preparation, respectively. Before analysis of the surface membrane determinants the cells were washed twice with a PBS solution (300 mosmol; pH 7.8) supplemented with 0.5% heat-inactivated BSA (Organon Teknika, Osa, The Netherlands).

Isolation of AM. All lavage studies were approved by the Medical Ethics Committee of the Erasmus University Hospital Dijkzigt, Rotterdam. BAL was performed in healthy, nonsmoking volunteers after premedication with thiazinamium and local anesthesia using a lidocaine 2% spray. The bronchoscope was placed in wedge position in the right middle lobe, and four aliquots of 50 ml sterile saline solution were infused and aspirated immediately in a siliconized specimen trap placed on melting ice. Immediately after collection the BAL fluid was strained through a sterile nylon gauze to trap large mucous particles, whereafter the BAL cells were isolated by centrifugation at 4°C for a force of 400 x g. More than 90% of the BAL cells appeared to be macrophages as judged by May-Grünwald Giemsa staining. Before analysis of the surface membrane determinants the cells were washed twice with PBS/0.5% BSA.

Cell lines. U937 cells, originally described by Sundström and Nilsson (23) were maintained at concentrations of 0.25 to 1.0 x 10^6/ml in RPMI 1640 medium (Flow Laboratories, Irvine, UK) supplemented with FCS (GIBCO, Paisley, UK) (10%), penicillin G sodium (100 U/ml), streptomycin (100 mg/ml), and gentamicin sulfate (0.1 mg/ml; Biochrom KG, Berlin, Germany). THP1 cells (24) were obtained from the American Type Culture Collection (Rockville, MD) and maintained according to their instructions. In some experiments, to culture U937 and THP1 cells under the same conditions as PBM or AM, cells were maintained in a modified Iscove’s medium (see below).

Culture of cells. Generally, U937 and THP1 cells were cultured as described above. In some experiments they were cultured as PBM and AM. PBM and AM were cultured in a modified Iscove’s medium (GIBCO) as described previously (25), in which BSA is replaced by human serum albumin supplemented with 5% autologous heat-inactivated serum. The culture medium was found to be endotoxin-free (defined as less than 1.0 ng/ml of endotoxin as quantified by the Limulus amoebocyte lysate assay). PBS (4 x 10^6/ml) and AM (1-2 x 10^6/ml) were cultured at 37°C, 5% CO2, and 100% humidity in Teflon bags (Janssen’s MNL, St.-Niklaas, Belgium) for 1 to 7 days. For detection of surface membrane determinants, cells were harvested after centrifugation. Subsequently, the cells were washed twice with PBS/0.5% BSA/0.5% sodium azide. The viability as determined by propidium iodide exclusion exceeded 95%. No significant differences in the numbers of cells recovered after culture in the presence of rIL-4 or anti-IL-4 antiserum could be observed.

rIL-4, rIL-2, and anti-IL-4 antiserum. Human rIL-4 was a generous gift of Dr. H. F. J. Savelkoul (Erasmus University, Rotterdam, The Netherlands) and Dr. K. Arai (DNAX Research Institute, Palo Alto, CA). A polyclonal rabbit anti-IL-4 antiserum was used. This antiserum blocks the biologic activity of IL-4, as has been described earlier (26). Human rIL-2 was obtained from Eurocetus (Amsterdam, The Netherlands).

Detection of surface membrane determinants. For immunofluorescence staining of PBM and AM the following mAb were used: CD13 (92-0 (IgG2a), Dr. C. E. van der Schoot, Amsterdam, The Netherlands); My7 (IgGl) Coulter Clone, Hialeah, FL); CD23 (IgGl, Bio-Rad, Cambridge, UK); and CD15 (IgG2a, Becton Dickinson, San José, CA). Isotype-matched antibodies which were directed against idiotypic determinants on a B cell lymphoma cell line and did not react with monocytes were used as control antibodies. Fifty microliters of the PBM or AM cell suspension (2 x 10^6/ml) were incubated for 50 min at 4°C with 50 μl of one of the optimally titrated mAb. After washing three times with 200-μl microtiter plates, the cells were resuspended in a solution of fluorescein isothiocyanate (FITC) conjugated goat anti-mouse antibody and returned to the same plates. After three washings the cells were incubated for 20 minutes at room temperature with a FITC-conjugated antibody. The fluorescence intensities of CD13 and CD23 expression on the corresponding cells were determined by a FACScan (Becton Dickinson). The fluorescence equivalent background. For immunofluorescent staining of U937 and THP1 cells, the cell suspension (2 x 10^6/ml) was preincubated for 10 min with a solution of fluorescein isothiocyanate (FITC) conjugated goat anti-mouse antibody. After this preincubation, interpretation of the immunofluorescent staining with the relevant mAb was troublesome because of high aspecific binding. After this preincubation, the cells were stained with the relevant mAb as described above for PBM and AM.

All standard washings were performed with PBS/0.5% BSA/0.5% sodium azide.

Preparation of total cellular RNA and Northern blot analysis. Total cellular RNA was isolated from U937 and THP1 cells and freshly isolated or cultured PBM and AM largely according to Chomczynski and Sacchi (27). Briefly, cells were washed twice in PBS. Cells (5-10 x 10^6) were homogenized with 0.5 ml of solution D in a 1.5 ml Eppendorf tube (5 x 1000 g, 4°C, 5 min). After homogenization, 0.5 ml of water-saturated phenol, and 0.1 ml of chloroform were added to the homogenate, with thorough mixing after the addition of each reagent. The final suspension was cooled on ice for 15 min. Samples were centrifuged at 10,000 x g for 15 min at 4°C. The aqueous phase was transferred to a fresh tube containing 0.5 ml of isopropanol, and then placed at 4°C for at least 6 h to precipitate RNA. RNA was recovered by centrifugation (10,000 x g, 15 min, 4°C), dissolved in 0.3 to 0.5 ml solution D, and precipitated again with 1 volume of isopropanol. After centrifugation (10,000 x g, 15 min, 4°C), the RNA pellet was washed in 0.5 to 1.0 ml 75% ethanol and eventually dissolved in 25 to 50 μl RNAase-free water. Total RNA (10 μg) was purified by electrophoresis in a 1 X agarose gel (28), subsequently vacuum transferred (29) onto a nylon membrane (NY-13 N; Schleicher and Schuell, Dassel, Germany), fixed to the membrane with a 254 nm UV cross-linker (Stratalinker, Stratagene, La Jolla, CA) and hybridized with the CD13 probe, which was labeled according to the Klenow-oligonucleotide method (30). Prehybridization and hybridization of RNA blots were performed as described elsewhere (28). After hybridization the blots were washed and exposed to Fuji NIF-RX films (Fuji Photo Film Co., Japan) with intensifying screens.

Preparation of CD13 cDNA probe. Total RNA was extracted from AM, which were obtained from a healthy individual. The cDNA was synthesized with reverse transcriptase (31) and subsequently amplified by using the PCR method as described elsewhere (32). For the PCR-mediated amplification, synthetic oligonucleotide primers were designed according to the published CD13 cDNA sequence (18). The CD13 upstream primer, acaggtaTCCAGAGGAGGAGATGTC, including an aspecific 5′-tandem (underlined) with an EcoRI restriction site, and the CD13 downstream primer, AgATGTGGTGGC, including an aspecific 5′-tandem (underlined) with an XbaI restriction site, were used in the PCR. The obtained 562-bp PCR product was electrophoresed in a 0.7%-agarose gel, and the band containing the probe was cut out of the gel. The probe was electrophoretically recovered from the agarose gel fragment by using a Bio-Rad Geneclean Kit (Bio-Rad, Hercules, CA) and cloned in the pUC19 vector with EcoRI and XbaI restriction enzymes. Sequence analysis of the cloned PCR fragment confirmed that the isolated probe represented the expected CD13 sequence.

Functional assay for aminopeptidase-N activity. The presence of aminopeptidase-N activity on PBM, U937, or THP1 cells was detected largely as described earlier (33). Briefly, the assay was performed in wells (in triplicate) of 96-well microtiter plates. Cells were washed twice in PBS. Fifty microliters of a cell suspension (1-3 x 10^6 cells/ml) were incubated with 50 μl of L-leucine-p-nitroanilide (Sigma) (8.56 mM) for 30 min at 37°C. After this incubation enzymatic reaction was stopped by adding a solution of 1 M sodium carbonate to give a final concentration of 35 μl of a 30% (v/v) acetic acid solution, and the increase in specific absorbance (at 405 nm as a result of accumulation of free p-nitroanilide) was determined by using a Titertek Multiskan MCC plate reader (I.C.N., Biomedicals B. V., Amsterdam, The Netherlands). Aminopeptidase-N activity was expressed as the production of p-nitroanilide in 30 min by 10^6 cells (nmol/30 min x 10^6 cells).

RESULTS

CD13 and CD23 surface expression on U937 cells, THP1 cells, PBM, and AM. U937 cells cultured in medium containing RPMI 1640 and 10% FCS do not express the CD13 Ag. On the other hand, THP1 cells and freshly isolated PBM or AM clearly express this Ag (Fig. 1). The CD23 Ag could not be demonstrated on either of these cell sources (data not shown).

Influence of IL-4 on expression of the CD13 Ag on...
human monocytes. Highly purified PBM were cultured for 3 days. As compared with the expression directly after isolation, the expression of the CD13 Ag was enhanced after culture (Fig. 2). This increase was consistently seen in all our experiments \((n = 7)\). It could only partially be explained by an increase in cell size as there was only a limited rise in forward light scatter signal. Besides this spontaneous increase in CD13 Ag expression upon culture, IL-4 caused a considerable further increase (Fig. 2). Compared with the control cultures, IL-4 induced a 2- to 3-fold further increase in CD13 Ag expression. This further increase was dependent on the concentration of IL-4 [maximal increase was found at a concentration of 50 U IL-4/ml] (data not shown) and could be blocked by the anti-IL-4 antiserum, adding proof to the specificity of the induction (Table I). PBM cultured in the presence of both IL-4 and the anti-IL-4 antiserum exhibited the same spontaneous but small up-regulation of CD13 Ag as PBM cultured in mock-medium (Table I). In addition, IL-2 did not induce a further increase in CD13 Ag expression (Table I). Because IL-4 is known to have long lasting (MHC class II Ag (9), CD14 Ag(3), RFD9 Ag(5) and transient (CD23 Ag (8)) effects on the expression of cell membrane determinants, we studied the kinetics of the influence of IL-4 on CD13 Ag expression.

**Influence of IL-4 on the expression of the CD13 Ag on human monocytes during a culture period of 7 days.** An up-regulation of the determinant recognized by CD13 was already observed after 1 day of culture (Fig. 3 and Table II). This increase was observed in medium without IL-4, but was much more substantial when PBM were cultured with IL-4. Maximal induction of CD13 Ag was seen after 2 to 3 days of culture. Thereafter, expression of CD13 Ag diminished. However, even after 7 days of culture expression of CD13 Ag was higher than the expression on freshly isolated PBM. During the whole culture period, PBM cultured in the presence of IL-4 exhibited a higher expression of CD13 Ag than PBM cultured in medium without IL-4.

We compared the transient induction of CD13 Ag by IL-4 with the known transient induction of FceRIIb (CD23) and the IL-4-induced down-regulation of CD14 Ag(3) (Table II). Also in our experiments we observed a transient induction of FceRIIb by IL-4. Maximal expression was seen after 2 to 3 days of culture, coinciding with the maximal induction of CD13 Ag expression. In contrast, down-regulation of CD14 Ag by IL-4 seemed to be a long lasting phenomenon. Expression of CD14 Ag was

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**Table I**

<table>
<thead>
<tr>
<th>Days of Culture</th>
<th>Medium</th>
<th>Medium + IL-2 (1000 U/ml)</th>
<th>Medium + IL-4 (100 U/ml)</th>
<th>Medium + IL-4 (100 U/ml) + anti-IL-4</th>
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<td>36.7</td>
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*a* Freshly isolated monocytes or monocytes cultured either in mock-medium or in the presence of IL-2, IL-4 alone, or both IL-4 and anti-IL-4 were labeled with Q20 (CD13) and FITC-conjugated GAM F(ab)\(_2\). A representative experiment out of seven is shown.

Data are expressed as relative fluorescence, i.e., the ratio between the mean linear fluorescence intensity of cells labeled with the relevant antibody and the mean fluorescence intensity of cells labeled with the isotype-matched control antibody.

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**Figure 1.** Expression of the CD13 Ag on human monocytic cell lines (U937 and THP1), freshly isolated PBM, and AM. The fluorescence intensity of 7500 cells was determined on a log scale with a FACScan. The left-hand histogram in each of the four panels represents cells stained with an IgG2a control antibody and FITC-labeled GAM F(ab)\(_2\), whereas the right-hand histogram represents cells stained with Q20 (CD13) and FITC-labeled GAM F(ab)\(_2\).

**Figure 2.** Effects of IL-4 on the expression of the CD13 Ag on blood monocytes. Cells were stained with Q20 (CD13) and FITC-labeled GAM F(ab)\(_2\) either directly after isolation (B), or after a culture period of 3 days in modified Iscove's medium without (C) or with (D; bold histogram) 100 U IL-4/ml. Histogram A represents monocytes stained with an IgG2a control antibody and FITC-labeled GAM F(ab)\(_2\). Fluorescence intensity of 7500 cells was determined on a log scale with a FACScan. A representative experiment out of seven is shown.

**Figure 3.** Kinetics of the effect of IL-4 on the membrane expression of CD13 Ag on human monocytes. Monocytes were cultured without (C) or with (D) IL-4 (100 U/ml). Fluorescence intensity of 7500 cells was determined with a FACScan. On the ordinate Is given the relative fluorescence, calculated as described in the footnote to Table I. Data of a representative experiment out of three are shown.
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Neither culture in RPMI 1640 supplemented with 10% FCS and rIL-4 nor culture in modified Iscove's medium supplemented with rIL-4 revealed induction of CD13 Ag expression on U937 cells. Although on PBM CD13 Ag expression increased after only 1 day of culture with 30 U IL-4/ml, induction of CD13 Ag expression on U937 cells could not be detected even after 2 to 3 days of culture with 100 U IL-4/ml.

Human AM were found to express CD13 Ag constitutively (Fig. 1). Upon culture, this expression increased, and this increase appeared to be maximal after 2 to 3 days of culture (Fig. 4). With respect to the kinetics of increase in CD13 Ag expression during culture, PBM and AM appeared to be comparable. Moreover, IL-4 caused a further increase in CD13 Ag expression similar to that observed in PBM (Figs. 4 and 5). Maximal expression was observed after 2 to 3 days of incubation. After 4 to 5 days, expression of CD13 Ag on AM cultured with IL-4 was almost identical to the expression on AM cultured without IL-4. However, expression after 4 to 5 days of culture was still higher than expression on freshly isolated AM. These data indicate that, besides the basal culture-induced increase in CD13 Ag expression, IL-4 induced an additional transient up-regulation of CD13 Ag on AM. On the other hand, IL-4 was unable to induce FcεRIIb expression on AM (Fig. 4). Because an IL-4-induced increase of CD13 Ag expression was observed only in constitutively CD13+ cells (PBM and AM) and not in constitutively CD13− cells (U937), we examined whether IL-4 was able to modulate expression on THP1 cells, which are, like U937 cells, immature monocytic cells but constitutively express CD13 Ag. In THP1 cells, expression of CD13 Ag was not changed, neither when these cells were cultured in RPMI 1640 supplemented with 10% FCS and rIL-4 nor when they were cultured in modified Iscove’s medium supplemented with rIL-4 (data not shown). On the other hand, THP1 cells were able to respond to IL-4, just as expression of FcεRIIb was induced on U937 cells (data not shown).

IL-4 increases LAP activity of human monocytes. To almost completely lost after 2 days of culture in the presence of IL-4 and reexpression was not observed even after 7 days of culture. This indicates that IL-4 has transient and sustained effects. The transient IL-4 effects probably reflect a temporary change in state of activation, whereas the long lasting effects may regard monocyte maturation.3

As there are indications that the maturation/differentiation state of the cell may determine its susceptibility to influences of IFN-γ or TNF-α (21), we investigated whether more mature and immature representatives of the mononuclear phagocyte system respond to IL-4 in the same way as PBM.

Influence of IL-4 on the expression of the CD13 Ag on U937 cells, THP1 cells, and human AM. U937 cells are known to express FcεRIIb upon culture in the presence with IL-4 (34). In our studies, we could also demonstrate induction of a weak expression of FcεRIIb by IL-4. This induction appeared to be transient and was maximal after 3 days of culture. On the other hand, culture with IL-4 did not result in induction of CD13 Ag (Fig. 4).

<table>
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<td>0.8</td>
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Table I. Time course of the influence of IL-4 on the expression of the CD13, CD14, and CD23 Ag on human blood monocytes.

*Monocytes were labeled with either Q20 (CD13), or UCHM1 (CD14), or 701 (CD23), followed by labeling with FITC-conjugated GAM F(ab)2 either directly after isolation or after 1, 2, 5, 6, or 7 days of culture with or without IL-4. A representative experiment out of three is shown.

Data are expressed as relative fluorescence, calculated as described in the footnote of Table I.

Not determined.

Figure 4. Time course of the influence of IL-4 on the membrane expression of the CD13 and CD23 Ag on the monocytic cell line U937 and human alveolar macrophages. Cells were cultured either without (O) or with (•) 30 U IL-4/ml. Fluorescence intensity of 7500 cells was determined on a log scale with a FACScan, A representative experiment out of three is shown.

Figure 5. Effects of IL-4 on the expression of the CD13 Ag on alveolar macrophages. Cells were stained with Q20 (CD13) and FITC-labeled GAM F(ab)2 either directly after isolation (B), or after a culture period of 2 days in modified Iscove’s medium without (C) or with (D: bold histogram) 30 U IL-4/ml. Histogram A represents macrophages stained with an IgG2a control antibody and FITC-labeled GAM F(ab)2. Fluorescence intensity of 7500 cells was determined on a log scale with a FACScan. A representative experiment out of three is shown.
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**TABLE III**

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Monocytes were assayed for their LAP activity directly after isolation and after 2 days of culture with or without 100 U of IL-4/ml. Monocytes of the same donor were also labeled with Q20 and FITC-conjugated GAM F(ab)₂ directly after isolation and after 2 days of culture with or without 100 U IL-4/ml.

*Data represent enzymatic activity and are expressed as the rate of accumulation of p-nitroanilide, nmol/30 min × 10⁶ cells.

Data are expressed as relative fluorescence, calculated as described in the footnote to Table I.

determine whether the IL-4-induced up-regulation of CD13 Ag reflects a functional increase in aminopeptidase activity of PBM, we studied whether IL-4 enhanced the capacity of PBM to degrade l-leucine-p-nitroanilide. PBM cultured for 2 days showed a decrease in LAP activity compared with freshly isolated PBM, despite an increase in CD13 Ag membrane expression (Table III). These apparent contradicting data may be explained by preliminary observations that also other membrane enzymes (which may be down-regulated upon culture) can display peptidase activity comparable to CD13 Ag (Dr. E. Fava­loro, personal communication). On the other hand, PBM cultured for 2 days with IL-4 exhibited a higher activity to degrade l-leucine-p-nitroanilide than PBM cultured without IL-4 (Table III). These data add proof that IL-4 is able to increase the functional activity of PBM to degrade l-leucine-p-nitroanilide. U937 cells, which do not express CD13 Ag, exhibited a 20-fold lower capacity to degrade l-leucine-p-nitroanilide, whereas THP1 cells, which do express CD13 Ag, had a capacity comparable with freshly isolated PBM (data not shown). To obtain more direct evidence for the influence of IL-4 on the expression of CD13 Ag and because IL-4 has been described to induce CD23 mRNA (35), we investigated the effect of IL-4 on CD13 mRNA.

IL-4 increases the expression of CD13 mRNA. Total RNA was isolated from freshly isolated human PBM and PBM cultured for 2 days with or without IL-4. Total RNA was separated by electrophoresis and hybridized with a ³²P-labeled CD13 cDNA probe. The results in Figure 6 show that IL-4 induced a strong increase in the level of CD13 mRNA in PBM (Fig. 6, lane 2). PBM cultured for 2 days in mock-medium exhibited a small, spontaneous increase in the level of CD13 mRNA (Fig. 6, lane 3). CD13 mRNA was not detectable in U937 cells, whereas the level of CD13 mRNA in THP1 cells resembled the level in freshly isolated PBM and AM.

**DISCUSSION**

Initially, it was thought that a certain cytokine directed its single activity at one particular cell type. Nowadays, it is generally accepted that different cytokines can regulate the same immune activity, that a single cytokine may effect a multitude of activities, and that these activities may be directed at multiple cell types (36-39). Moreover, recent reports describe that the responsiveness of a certain cell type to a particular cytokine may depend on the stage of maturation/differentiation of that cell type (21). IL-4 was originally described as a B cell growth factor, but is now also known to influence T lymphocytes, NK cells, and monocytes as well. Some of the many effects of IL-4 can also be brought about by other cytokines (38, 39). The data presented here widen the range of activities of IL-4 and show that IL-4 responsiveness of mononuclear cells depends on their stage of maturation.

It has been reported by different investigators that IL-4 is able to modulate the expression of several cell surface determinants. IL-4 is able to induce a transient expression of FcγR (CD23) on PBM (8) and monocytic cell lines (35). Furthermore, IL-4 enhances the expression of class II MHC Ag on PBM (9) and AM (P. Th. W. van Hal, unpublished observations), down-regulates the expression of FeRIIb on PBM (40), and modulates maturation markers on PBM. Our data indicate that IL-4 is also able to increase the expression of the CD13 Ag. This increase appears to be transient and resembles the kinetics of IL-4-induced FcγR expression. Furthermore, IL-4 increases the level of CD13 mRNA and raises the capacity to degrade l-leucine-p-nitroanilide. Besides these IL-4-induced increases, a small and spontaneous up-regulation of CD13 Ag was observed after culture of monocytes in mock-medium. It is unlikely that this spontaneous up-regulation is due to contaminating amounts of IL-4, as culture of PBM in the presence of both IL-4 and the anti-IL-4 antiserum reduced CD13 Ag expression to the levels of PBM cultured in mock-medium, and not to the levels of freshly isolated PBM. Furthermore, this spontaneous increase could not be explained by serum components or adherence, as culture of PBM in serum-free medium gave similar results (data not shown), and PBM were cultured under nonadherent conditions in Teflon bags, respectively. As culture of PBM in mock-medium induces also a spontaneous increase in MHC class II expression (9), it appears that in our system activation takes place, most likely because of medium components, e.g., low levels of LPS. The spontaneous increase in CD13 Ag expression may be part of this activation.

To our knowledge this is the first report on IL-4-induced expression of CD13 Ag, although recently Rohrbach and Conrad (41) reported that leucine-aminopeptidase (EC 3.4.11.2 and identical to CD13 Ag) activity in cultured PBM was enhanced by the presence of T lymphocytes. They found that the majority of increase occurred during the first day of culture, whereas they saw a further slight increase up until day 6 of culture. Although they did not confirm their data with immunofluorescence staining for CD13 Ag and did not speculate on the mechanism by which leucine-aminopeptidase activity was increased, we think that their results can be explained by the production of IL-4 by activated T lymphocytes, which is consistent with our own results.

Surprisingly, induction of CD13 Ag by IL-4 could not
IL-4 REGULATES EXPRESSION OF CD13 AND CD23 ON MONOCYTES/MACROPHAGES


Figure 6. Induction of CD13 mRNA by IL-4 in human PBMs. Total RNA was isolated from freshly isolated monocytes (lane 1), and from cells cultured for 2 days in the presence (lane 2, 4) or absence (lane 3, 5) of IL-4. Each of these three lanes contains 10 ng of total RNA. Lanes 4 to 9 contain 1, 2, 4, 8, 16, and 32 ng of RNA from the CD13+ cell line THP1, respectively. Lanes 10 and 11 contain 10 and 20 ng of RNA from the CD13+ cell line U937, respectively. Lane 12 contains 5 ng of total RNA from freshly isolated AM, RNA was separated by electrophoresis, transferred onto a nylon membrane, and hybridized with the 32P-labeled CD13 cDNA probe (upper panel). The lower panel shows ethidiumbromide staining of the gel after electrophoresis to visualize loading per lane.

be achieved on immature monocytic cell lines, such as U937 and THP1. We confirmed the earlier observations that IL-4 is able to induce FcεRIIb expression on these cell lines. Therefore, unresponsiveness to IL-4 concerning the induction of CD13 Ag cannot be explained as a result of absence of functional IL-4R. On the other hand, AM, just like PBM, increased their expression of CD13 Ag upon culture with IL-4. AM and PBM constitutively express CD13 Ag, whereas U937 cells lack this Ag. Therefore, one could speculate that IL-4 is only able to enhance actual expression of CD13 Ag, although it is unable to induce expression on a CD13+ cell type. If so, one would expect an increase in expression of CD13 Ag on THP1 cells, which are constitutively positive for this marker, upon culture with IL-4. This, however, could not be demonstrated in our experiments. We therefore propose, that immature monocytic cells are unable to enhance CD13 Ag expression upon culture with IL-4 despite the presence of functional IL-4R, and that the ability to enhance CD13 Ag expression after culture with IL-4 is acquired only upon maturation. On the other hand, it may also be possible that the cell lines in question are unable to up-regulate CD13 Ag because of cell line-related properties other than immaturity, e.g., altered state of (de)activation.

An analysis of the literature showed that others also indicated that cytokine effects are variable depending on the cell type and/or the stages of differentiation. Kawabe et al. (34) mentioned that CD23 Ag could be up-regulated by IL-4 on normal B lymphocytes, but not on EBV-transformed B lymphoblastoid cell lines. Littman et al. (39) found that IL-4 did not stimulate undifferentiated HL-60 cells to produce C2. However, when these cells were first preincubated with vitamin D3 to induce monocyte differentiation, they did produce C2 in response to IL-4. Recently, Watanabe and Jacob (21) demonstrated that TNF-α enhanced IFN-γ-induced MHC class II Ag expression in immature cells, such as U937 cells, whereas TNF-α had no effect on IFN-γ-induced MHC class II Ag expression in 12-O-tetradecanoylphorbol-13-acetate-differentiated U937 cells. They also proposed that cytokine effects depend on the maturation stage of cells.

As IL-4 was able to induce FcεRIIb expression on U937 cells and PBM, we were surprised to see that it was unable to induce expression on AM. Although there are some reports showing expression of FcεRIIb on AM (42, 43), it seems unlikely from our study that IL-4 plays a decisive role in this expression, at least as an isolated factor. Bieber et al. (44) were able to induce FcεRIIb on a subpopulation of human epidermal Langerhans cells, but they used IL-4 in a high dose (1000 U/ml) and in combination with IFN-γ (1000 U/ml). However, it should be emphasized that we cannot exclude the possibility that IL-4 in combination with other cytokines may be able to induce FcεRIIb on AM, or that IL-4 is able to induce FcεRIIb on a small (immature) subpopulation of AM, as FACScan analysis is inappropriate for detecting positive subpopulations constituting less than 1–2% of the total cell population. Nevertheless, virtually the whole population of U937 cells and PBM express FcεRIIb after culture with IL-4, whereas virtually the whole population of AM does not respond. These findings suggest that upon maturation the ability to express FcεRIIb during culture with IL-4 is lost. This notion is supported by the findings of Te Velde et al. (45), who demonstrated that IL-4-matured PBM do not reexpress FcεRIIb upon renewed addition of IL-4 on day 4 of culture.

In conclusion, our findings reveal a new property of IL-4, namely an up-regulation of the expression of CD13 Ag on monocytic cells. As the CD13 Ag represents aminopeptidase-N activity, this up-regulation may be part of an IL-4-induced activation of monocytic cells. Furthermore, our findings provide a model to explain the paradoxical effects of cytokines on a certain cell type. Positive or negative effects of a cytokine on a single cell type may be related to differences in stage of maturation, differentiation, or activation.

Acknowledgments. We gratefully acknowledge Prof. Dr. R. Benner for his continuous support, Dr. T. Breit and Dr. R. van Ommen for their advice in preparation of the CD13 cDNA probe, Mr. R. J. F. Huijbens for isolation of human monocytes, Mr. T. M. van Os for excellent assistance in preparing the figures, Mr. A. Prins for assisting in Northern blot analysis, and Mrs. H. J. Elsenbroek-de Jager and Ms. G. De Korte for their skillful secretarial help.

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