Potential Indirect Anti-Inflammatory Effects of IL-4

Stimulation of Human Monocytes, Macrophages, and Endothelial Cells by IL-4 Increases Aminopeptidase-N Activity (CD13; EC 3.4.11.2)¹


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IL-4 up-regulates various monocytic properties that are associated with pro-inflammatory functions. Paradoxically, IL-4 may also act as an anti-inflammatory agent by down-regulating the production of several inflammatory mediators. As the activity of some mediators has recently been shown to be regulated by peptidases, we examined whether IL-4 was able to modulate the expression of a cell membrane-associated peptidase, aminopeptidase-N (CD13). IL-4 caused a dose-dependent increase in the expression of CD13 Ag on highly purified human blood monocytes. Maximal expression was observed around 48 h of culture. This IL-4-induced increase was completely blocked by anti-IL-4 antiserum. Furthermore, the increase in surface expression was preceded by increased mRNA levels of CD13, which was maximal around 24 h of culture. We also observed that CD13-mediated leucine-aminopeptidase activity of monocytes was induced by IL-4. Other CD13-expressing cells were also sensitive to IL-4, as CD13 Ag expression and CD13 mRNA levels were up-regulated in human alveolar macrophages and endothelial cells upon IL-4 treatment. The increased expression of cell membrane aminopeptidase-N represents a potentially increased cellular ability to inactivate inflammatory mediators. Therefore, these findings represent further evidence of IL-4-mediated anti-inflammatory actions. We postulate that up-regulation of aminopeptidase-N expression may be an indirect mechanism of IL-4 to modulate the action of bioactive peptides. This mechanism may underlie, at least partially, the anti-inflammatory effects of IL-4 in vivo. The Journal of Immunology, 1994, 153:2718.

IL-4 was originally characterized by its ability to effect differentiation of resting B lymphocytes (1). More recently, it has been shown that IL-4 may also display a wide range of effects on several cell types (2). It can act as a growth factor for activated T lymphocytes, thymocytes, NK cells (3–5), and mast cells (5, 6). In addition, monocytic cells can also be influenced by IL-4, which is reported to induce monocyte maturation³ (7). Furthermore, some of the actions of IL-4 on mononuclear cells are considered to be stimulatory. IL-4 increases expression of MHC class II Ag (7), increases antigen-presenting capacity (8), and induces FceRIIb expression (9). IL-4 appears also to inhibit some parameters of monocyte activation. Thus, IL-4 inhibits superoxide production (10), inhibits the release of PGE2 (11), IL-1β (11–14), IL-6 (12, 15, 16), IL-8 (17), TNF-α (11–13), and up-regulates the production of IL-1R antagonist (14, 18). Based upon the latter findings, it was suggested that IL-4 may have anti-inflammatory properties (11–19).

During our studies on the influence of cytokines on monocytes and macrophages, we observed that IL-4 was

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also able to modulate the expression of aminopeptidase-N. Initially, this enzyme (EC 3.4.11.2, gp150, CD13) was designated as a marker for subpopulations of hemopoietic cells (20–23). Currently, it is generally accepted that many other cell types (including fibroblasts, the renal tubular and intestinal epithelium (24, 25), endothelial cells (EC)\(^2\)(26, 27), and synaptic membranes of cells of the central nervous system (28)) express this enzyme. It is hypothesized that aminopeptidase-N also plays an important role in modulating the activity of bioactive oligopeptides, comparable to the role of neutral endopeptidase (EC 3.4.24.11) (29–32). Accordingly, aminopeptidase-N may play a role in regulation of inflammatory and immunologic responses. Modulation of the expression of aminopeptidase-N may therefore influence these oligopeptide-mediated responses. In this report, we demonstrate that IL-4 is able to up-regulate the expression of CD13 Ag on monocytic cells. Up-regulation is also seen in non-monocytic cells (in casu, endothelial cells), although this was less pronounced. This up-regulation is accompanied by an increase in aminopeptidase-N, therefore influence these oligopeptide-mediated responses. Modulation of the expression of aminopeptidase-N may therefore influence these oligopeptide-mediated responses.

**Materials and Methods**

**Isolation of PBM**

Human PBM were isolated from 500 ml blood from healthy volunteers as described elsewhere (33). Briefly, mononuclear cells were separated by density centrifugation with a blood component separator. Next, the mononuclear cells were fractionated into lymphocytes and monocytes by centrifugal elutriation. The monocyte preparation was over 95% pure as judged by May-Grünwald Giemsa staining and contained more than 98% viable cells as judged by trypan blue exclusion. Lymphocytes and granulocytes constituted less than 2% and 3% of the monocyte preparation, respectively. Before analysis of the surface membrane determinants, the cells were washed twice with a PBS solution (300 mM; pH 7.8) supplemented with 0.5% heat-inactivated BSA (Organon Teknika, Oas, The Netherlands). Before isolation of total RNA or detection of LAP activity, the cells were washed twice with a PBS solution.

**Isolation of AM**

All lavage studies were approved by the Medical Ethics Committee of the Erasmus University/University Hospital Dijkzigt, Rotterdam. BAL was performed in healthy, nonsmoking volunteers after premedication with thiazinamium and local anesthesia using a lidocaine (2%, w/v) 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 mucus particles, after which the BAL cells were immediately in a siliconized specimen trap placed on melting ice. 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 mucus particles, after which the BAL cells were isolated by centrifugation at 4°C at 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. Before isolation of total RNA or detection of LAP activity, the cells were washed twice with a PBS solution.

**Isolation of EC**

Human EC were isolated from umbilical cord vein by collagenase digestion as described elsewhere (34).

**Cell lines**

THP-1 cells (35) were used as control cells because of their CD13 mRNA and CD13 Ag expression. They were obtained from the American Type Culture Collection (Rockville, MD) and maintained according to their instructions.

rIL-4, rIL-2, rIFN-γ, GM-CSF, LPS, and anti-IL-4 antisera

Human rIL-4 was a generous gift of Dr. H.F.J. Savelkoul of our department and Dr. K. Arai (DNAX Research Institute, Palo Alto, CA). Human rIL-2 was obtained from Eurocetus (Amsterdam, The Netherlands). Human rIFN-γ (specific activity 10\(^7\) U/mg) and human rGM-CSF (specific activity 10\(^9\) U/mg) were kindly provided by Drs. P. Trotna and S. Nagabhushan (Schering-Plough Corp, Bloomfield, NJ). LPS (E. coli 0127:B8) was purchased from Difco Laboratories (Detroit, MI). A polyclonal rabbit anti-IL-4 antiseraum was used to block the biologic activity of IL-4, as has been described elsewhere (36).

**Culture of cells**

PBM and AM were cultured in a modified Iscove's medium (Life Technologies, Inc., Paisley, U.K.) as described elsewhere (37), in which BSA was replaced by human serum albumin. In some experiments this medium was supplemented with 2% autologous heat-inactivated serum. The culture medium was found to be endotoxin free (defined as less than 1.0 ng endotoxin/ml as quantified by the Limulus amebocyte lysate assay). PBM (2×10\(^7\) cells/ml and AM (1–2×10\(^7\) cells/ml) were cultured at 37°C, 5% CO\(_2\), and 100% humidity in Teflon bags (Janssen's MNL, St-Niklaas, Belgium) for 1 to 7 days. For detection of surface membrane determinants, isolation of total RNA, or detection of LAP activity, cells were harvested from the Teflon bags and separated from the culture supernatant by centrifugation. Subsequently, the cells were washed twice with either PBS/0.5% BSA/0.5% sodium azide when cells were used for detection of surface membrane determinants, or PBS when cells were used for isolation of total RNA or detection of LAP activity. The viability as determined by propidium iodide or trypan blue exclusion exceeded 85%. The numbers of cells recovered after culture in the presence of rIL-4, rIL-2, or anti-IL-4 antiseraum did not differ significantly.

EC were cultured in flat-bottom 6-well plates as described elsewhere (38). For detection of CD13 Ag expression, plates were washed twice with PBS (37°C) to remove nonviable, nonadherent EC. Thereafter, EC were incubated (5 min, 37°C) with a collagenase solution (Worthington, Biochemical Corporation, NJ) to harvest adherent cells. From this point, EC were handled as PBM and AM. For isolation of total RNA, plates were washed twice with PBS (37°C). Thereafter, adherent cells were homogenized with 0.5 ml of solution D and transferred to 1.5-ml Eppendorf tubes. From this point, EC were handled as PBM and AM.

**Detection of surface membrane determinants**

For immunofluorescence stainings of PBM, AM, and EC the following mAbs were used: CD13 (Q20/1gG2a), Dr. C. E. van der Schoot, CLB, Amsterand, The Netherlands; My7 (1gG1), Coulter Clone, Hialeah, FL; CD23 (Tul/1gG1), Biotest, Dreieich, Germany; Leu-20 (1gG1), Becton Dickinson, San Jose, CA). Isotype-matched control Ads were directed against idiotypic determinants on a B cell lymphoma cell line and did not react with monocytes, macrophages, or endothelium. Fifty microliters of the EC, PBM, or AM cell suspension (2×10\(^5\) cells/ml) were incubated for 30 min at 4°C with 50 μl of one of the (optimally titrated) mAbs. Incubation was performed in U-bottom 96-well microtiter plates. After three washings the cells were incubated with FITC-labeled GAM F(ab')\(_2\) (De Beer Medicinals B.V., Hilvarenbeek, The Netherlands) for 30 min at 4°C. After another three washings the cell pellets were resuspended for analysis of the fluorescence intensity by means of a FACScan (Becton Dickinson). The fluorescence intensities of Ag expression are expressed as a ratio relative to the background fluorescence intensity of cells stained.
with an isotype-matched control Ab. A value of 1.0 reflects fluorescence equivalent to background. When fluorescence intensity was determined on cultured cells, background fluorescence was determined on similarly cultured cells.

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 THP-1 cells, freshly isolated or cultured PBM and AM, and cultured EC largely according to Chomczynski and Sacchi (39). Briefly, cells (5–10 × 10^6) were homogenized with 0.5 ml of solution D in a 1.5-ml Eppendorf tube. Sequentially, 50 μl of 2 M sodium acetate (pH 4.0), 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 × g for 15 min at 4°C. The aqueous phase was transferred to a fresh tube containing 0.6 ml of isopropanol, and then placed at 4°C for at least 6 h to precipitate RNA. RNA was recovered by centrifugation (10,000 × 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 × g, 15 min, 4°C), the RNA pellet was washed in 0.6 to 1.0 ml 75% ethanol and eventually dissolved in 0.3 to 0.5 ml solution D, and precipitated again with 1 volume of isopropanol, and then placed at 4°C for at least 6 h to precipitate RNA. Total RNA (5–20 μg) was separated by electrophoresis in a 1% agarose gel (40) subsequently vacuum transferred (41) onto a nylon membrane (NY-13 N; Schleicher & Schuell, Dassel, Germany) fixed to the membrane with a 254 nm UV crosslinker (Stratalinker, Stratagene, La Jolla, CA), and hybridized with a CD13 probe, which was labeled according to the Klenow oligonucleotide method (42). The CD13 cDNA probe was prepared as described elsewhere (43). Prehybridization and hybridization of RNA blots were performed as described elsewhere (40). After hybridization the blots were washed and exposed to Fuji NIP-KX films (Fuji Photo Film Co., Tokyo, Japan) with intensifying screens. The quality and the amounts of RNA applied were controlled by rehybridization of the blots with a GAPDH probe. This probe was a 0.7-kb EcoRI - Pstl fragment (44).

**Semi-quantification of CD13 mRNA levels**

The relative density for bands on autoradiograms was estimated by laser scanning densitometry (video densitometer model 620, Bio-Rad Laboratories, Richmond, CA). Each gel containing RNA from kinetic IL-4 experiments with PBM, AM, or EC also contained a series of seven 1:2 dilutions (0.5–32 μg) of total RNA isolated from CD13 mRNA* THP-1 cells. As the linear relationship between band density on autoradiograms and the quantity of CD13 mRNA is only limited, we related the density of a particular CD13 band from PBM, AM, or EC to bands of comparable density in the dilution series of CD13 bands from THP-1 cells. In this way, it was possible to quantitate, indirectly but in a linear way, CD13 bands of different density from PBM, AM, or EC, whether or not they were exposed to IL-4 during different periods of time. CD13 mRNA levels are expressed in units, where a value of X represents the level of CD13 mRNA (present in the quantity of electrophorized total RNA) that is identical to the level of CD13 mRNA in X μg of THP-1 total RNA. If X is greater than the quantity of electrophorized total RNA, the particular cell sample contained relatively more CD13 mRNA than THP-1 cells. The same procedure was applied to estimation of GAPDH band densities. For final interpretation of CD13 mRNA levels, i.e., to compensate for differences in RNA loading per lane or culture-induced influences on general mRNA levels, CD13 mRNA levels were related to GAPDH mRNA levels.

**Functional assay for LAP-activity**

The presence of aminopeptidase-N activity on PBM was detected largely as described elsewhere (45). Briefly, the assay was performed in triplicate in flat-bottom wells of 96-well microtiter plates. Fifty microliters of a cell suspension (0.5 – 1.0 × 10^6 cells/ml) were incubated with 50 μl of l-leucine-p-nitroanilide (Sigma Chemical Co.) (8.36 mM) for 30 min at 37°C. After this incubation enzymatic activity was blocked by addition of 35 μl of a 30% (v/v) acetic acid solution. 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 × 10^6 cells).

In some experiments cells were pre-incubated with the CD13 mAb WM15 (IgG1, Dr. E. J. Favaloro, Westmead, Australia) for 30 min at 4°C to block CD13-mediated LAP-activity. As a control procedure, cells were pre-incubated with an isotype-matched control Ab. After this pre-incubation, cells were directly assayed for their LAP-activity as described above.

**Results**

**IL-4 up-regulates CD13 Ag expression on human monocytes**

Freshly isolated PBM expressed CD13 Ag. However, even after one day of culture, expression of CD13 Ag was found to increase significantly upon IL-4 treatment (Fig. 1, closed circles). This increase was seen consistently in all experiments (n = 9). Maximal increase was observed around day 2 of culture. To a limited extent, CD13 Ag expression was also enhanced in control cultures of PBM (Fig. 1, open circles). These findings could not be explained by an increase in cell size, as there was only a
Table I. Influence of IL-4 on the expression of the CD13 Ag on blood monocytes can be blocked by anti-IL-4 antiserum

<table>
<thead>
<tr>
<th>Days of Culture</th>
<th>Medium</th>
<th>Medium + IL-4 (100 U/ml)</th>
<th>Medium + IL-4 (100 U/ml) + Anti-IL-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>17.3(^b)</td>
<td>17.3</td>
<td>17.3</td>
</tr>
<tr>
<td>3</td>
<td>35.2</td>
<td>94.8</td>
<td>34.3</td>
</tr>
</tbody>
</table>

\(^a\) Freshly isolated monocytes or monocytes cultured either in control medium or in the presence of 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.

\(^b\) Data are expressed as relative fluorescence, i.e., the ratio between the mean linear fluorescence intensity of cells labeled with the relevant Ab and the mean fluorescence intensity of cells labeled with the isotype-matched control Ab.

limited rise in forward light scatter signal after 2 days, and CD13 Ag expression decreased upon further culture although cell size increased gradually. After more than 2 days of culture, expression of CD13 Ag diminished, but even after 7 days of culture expression was higher than the expression on freshly isolated PBM or PBM cultured in medium without IL-4. This IL-4-induced increase was dependent on the concentration of IL-4 and could be blocked by anti-IL-4 antiserum (Table I). We also studied whether other cytokines were able to affect CD13 Ag expression comparably to IL-4. Therefore, we cultured PBM for 2 or 3 days with either IL-2, IFN-\(\gamma\), GM-CSF or LPS. Neither LPS nor these cytokines were able to induce any additional increase in CD13 Ag expression as compared with the spontaneous increase observed when cells were cultured in control medium (Table II). Taken together, these results suggest that the transient increase in CD13 Ag expression upon culture with IL-4 is specific for IL-4.

IL-4 increases CD13 mRNA levels in human monocytes

To investigate whether increased CD13 Ag expression required enhanced mRNA levels, total RNA was prepared from freshly isolated PBM, and PBM after culture without or with IL-4. Total RNA was size-separated by electrophoresis and hybridized with the \(^32\)P-labeled CD13 cDNA probe and, at a later point of time, the \(^32\)P-labeled GAPDH cDNA probe. Analysis of the CD13 mRNA levels showed that culture of PBM with IL-4 caused a consistent and marked additional increase in CD13 mRNA level, as compared with the spontaneous increase observed when cells were cultured in control medium (Table II). Taken together, these results suggest that the transient increase in CD13 Ag expression upon culture with IL-4 is specific for IL-4.
Influence of IL-2, IFN-γ, GM-CSF, and LPS on the expression of the CD13 Ag on blood monocytes as compared with the influence of IL-4.*

<table>
<thead>
<tr>
<th>Days of Culture</th>
<th>Medium</th>
<th>Medium + IL-2</th>
<th>Medium + IFN-γ</th>
<th>Medium + GM-CSF</th>
<th>Medium + LPS</th>
<th>Medium + IL-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>39.3b</td>
<td>39.3</td>
<td>39.3</td>
<td>39.3</td>
<td>39.3</td>
<td>39.3</td>
</tr>
<tr>
<td>3</td>
<td>59.1</td>
<td>61.6</td>
<td>24.9</td>
<td>48.9</td>
<td>55.4</td>
<td>143.6</td>
</tr>
</tbody>
</table>

*Freshly isolated monocytes or monocytes cultured either in control medium or in the presence of one of the indicated cytokines (IL-2, IFN-γ, GM-CSF, IL-4) or LPS were labeled with Q20 (CD13) and FITC-conjugated GAM Fab'. A representative experiment out of three is shown.

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

Influence of IL-4 on the expression of CD13 Ag and CD13 mRNA levels in cultured human AM

We have demonstrated earlier that freshly isolated AM express CD13 Ag (43). Upon culture, a spontaneous and transient increase in CD13 Ag expression was observed, comparable with PBM. Likewise, IL-4 induced a more substantial increase in CD13 Ag expression with a maximum around day 2 of culture (Fig. 4, right panel). We studied also the kinetics of the effect of IL-4 on CD13 mRNA levels in AM. As in PBM, we observed an IL-4-induced rise in the level of CD13 mRNA. This increase was significantly larger than the spontaneous increase that was seen upon culture without IL-4 (Fig. 5, lanes 2 and 3). Furthermore, this increase was already seen after 5 h of culture and was maximal after 1 day of culture. The level of CD13 mRNA decreased upon further culture. After 4 days of culture, the level of CD13 mRNA in AM cultured with IL-4 did not differ any more from the level in AM cultured without IL-4 (Fig. 5, lanes 8 and 9).

Influence of IL-4 on the expression of CD13 Ag and CD13 mRNA levels in cultured human EC

In our experiments we could confirm, as has been demonstrated elsewhere (26, 27), that human endothelial cells in in vitro culture expressed CD13 Ag. After only 13 h of culture in the presence of IL-4 (100 U/ml), we found already a slight increase in the expression of CD13 Ag (relative fluorescence increased from 100 ± 11% to 122 ± 7% (n = 2)). Maximal increase of CD13 Ag expression was observed between 24 and 48 h of culture. The left-hand panel of Figure 4 shows the maximal IL-4-induced expression of CD13 Ag of one representative experiment (relative fluorescence increased from 7.9 (100%) to 18.8 (238%)). Incubation for more than 48 h resulted in a decrease of the expression of CD13 Ag. Therefore, the transient induction of CD13 Ag expression in human EC resembled the kinetics observed in our experiments with PBM and AM. Moreover, IL-4 also increased CD13...
Table III. **Time course of the influence of IL-4 on the level of CD13 mRNA in human PBM**

<table>
<thead>
<tr>
<th>Days of Culture</th>
<th>Medium</th>
<th>Medium + IL-4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD13 mRNA level</td>
<td>GAPDH mRNA level</td>
</tr>
<tr>
<td>0</td>
<td>&lt;2(^b)</td>
<td>5.2(^c)</td>
</tr>
<tr>
<td>1</td>
<td>34.2</td>
<td>6.8</td>
</tr>
<tr>
<td>2</td>
<td>7.2</td>
<td>3.1</td>
</tr>
<tr>
<td>5</td>
<td>&lt;2</td>
<td>4.7</td>
</tr>
</tbody>
</table>

\(^a\) Total RNA was prepared from freshly isolated PBM and PBM cultured in medium either without or with 100 U IL-4/ml. After fractionation of 5 \(\mu\)g RNA in an agarose gel, transfer to a nylon membrane, hybridization with the CD13 or GAPDH cDNA probe, and exposure to a film, the relative radioactivity for bands on the autoradiogram was estimated by laser scanning densitometry. Results in this table are derived from the autoradiograms shown in Figure 2. A representative experiment out of three is shown.

\(^b\) Data represent expression of CD13 mRNA in PBM relative to the expression in THP-1 cells. A value of \(X\) represents the level of CD13 mRNA present in the quantity of total RNA used for electrophoresis (in this case, 5 \(\mu\)g), which is identical to the level of CD13 mRNA present in \(X\) \(\mu\)g of total RNA isolated from THP-1 cells (see Materials and Methods for further explanation).

\(^c\) Data represent expression of GAPDH mRNA in PBM relative to the expression in THP-1 cells. Interpretation of the data is identical to the interpretation as described in footnote b.

\(^d\) Data represent the ratio between the CD13 mRNA (footnote b) and GAPDH mRNA (footnote c) levels.

FIGURE 3. Kinetics of the influence of IL-4 on the LAP activity of human PBM. Monocytes were assayed for their LAP activity directly after isolation (0 h of culture) and after culture without (O) or with (●) 100 U IL-4/ml. Total LAP activity (panels A and C) represents enzyme activity of PBM that were pre-incubated with an IgG1 control Ab. CD13-specific LAP activity (panels B and D) is calculated by subtracting the remaining LAP activity of PBM that were pre-incubated with the mAb WM-15 from the total LAP activity, and assumes that after pre-incubation with WM-15 the only aminopeptidase activity detected is that which is not mediated by CD13 Ag.

mRNA levels in EC comparable with the increase seen in PBM and AM (Fig. 6).

**Discussion**

Although IL-4 was initially described as a B cell stimulatory factor, it is now also known to have regulatory activity on human monocyte and macrophage functions. IL-4 may activate PBM to express FceRIIb and MHC class II Ag (7–9), and may thus contribute to inflammatory processes. Paradoxically, IL-4 has also been reported recently to display anti-inflammatory functions. The monocyte production of several cytokines and reactive oxygen species is suppressed by IL-4 (10–18), and the capacity to lyse tumor cells and microorganisms is decreased (10, 46, 47). Furthermore, IL-4 may control the accumulation of monocytes in areas of inflammation by enhancing apoptosis in
Influence of IL-4 on the expression of CD13 Ag on human EC and AM. The left-hand panel represents EC that were stained with CD13 mAb and FITC-labeled GAM F(ab')₂ either after a culture period of 2 days in medium without (C) or with (D; bold histogram) 100 U IL-4/ml. Histogram A represents EC stained with an IgG2a control Ab and FITC-labeled GAM F(ab')₂. One representative experiment out of two is shown. The right-hand panel represents AM that were also stained after a culture period of 2 days in medium without (C) or with (D; bold histogram) 100 U IL-4/ml. Histogram A represents AM stained with an IgG2a control Ab and FITC-labeled GAM F(ab')₂, whereas histogram B represents staining of freshly isolated AM with CD13 mAb and FITC-labeled GAM F(ab')₂. One representative experiment out of seven is shown. In both the left-hand and right-hand panels, fluorescence intensity of 7500 cells was determined on a log scale with a FACScan.

stimulated monocytes (19). Evidence that IL-4 may exhibit anti-inflammatory properties is accumulating and the first results of the use of IL-4 in vivo have been published recently (48, 49).

In the present study, it is shown that IL-4 up-regulates the expression of CD13 Ag on monocytes and macrophages, and thus may be a newly described mechanism modulating inflammation. The transient induction of CD13 Ag by IL-4 resembles the earlier reported IL-4-induced up-regulation of FceRIIb on PBM (9, 50). Maximal induction was seen after 2 days of culture. Kinetics of the IL-4-induced up-regulation of CD13 mRNA revealed maximal levels after 1 day of culture, indicating that peak mRNA levels preceded peak CD13 Ag expression. The increased CD13 mRNA levels may result from either IL-4-increased transcription or mRNA stability. Future nuclear run-off transcription experiments and mRNA half-life experiments may clarify this point. Indirect evidence that most IL-4 effects occur at the transcriptional level comes from other studies that described influences of IL-4 on mRNA transcription of IL-1Ra (51), IL-2 (52), IL-6, CD23, and type I and III procollagen.

Functionally, we observed an IL-4-induced increase in LAP activity more or less coinciding with the IL-4-induced expression of CD13 Ag. Although PBM cultured in control medium also exhibited a slight, spontaneous increase in CD13 Ag expression and CD13 mRNA level, this was not accompanied by an increase in LAP activity in our experiments. This may be a result of a negative influence on the optimal functional enzyme structure when cells are isolated from the in vivo environment and transferred to the artificial in vitro culture conditions. Nevertheless, IL-4-treated PBM exhibited a higher LAP activity than untreated PBM, indicating that IL-4 is able to increase the capacity of PBM to degrade aminopeptidase substrates. Not all LAP activity appeared to be a result of the presence of CD13 Ag, as the mAb WM-15 was unable to block LAP activity totally. This was demonstrated earlier (53) and may indicate that other cell membrane enzymes also display aminopeptidase-N-like activity. A more specific assay for measuring peptidase activity resulting from aminopeptidase-N-activity (which is not available yet) would address this issue appropriately.

PBM cultured in control medium exhibited a slight, spontaneous increase in CD13 mRNA and CD13 Ag. These increases in expression are unlikely to be caused by contaminating amounts of IL-4, as culture of PBM in the presence of both IL-4 and anti-IL-4 antiserum reduced CD13 Ag expression to the levels of PBM cultured in control medium, and not to the levels of freshly isolated PBM. Furthermore, this spontaneous increase could not be explained by either serum components or LPS or cellular adherence, as culture of PBM under serum-free conditions gave similar results, culture in the presence of LPS did not result in a more pronounced increase, and PBM were cultured under nonadherent conditions in Teflon bags.

Recently, it was described that activity of cell membrane peptidases could be modulated by cellular environmental factors. Werfel et al. demonstrated that the cell membrane expression of CD13 Ag was rapidly increased on PBM upon stimulation with C5a (54). This
Kinetics of the expression of CD13 mRNA in cultured alveolar macrophages

Kinetics of the expression of CD13 mRNA in cultured endothelial cells

FIGURE 5. Kinetics of the IL-4-induced up-regulation of CD13 mRNA expression in human AM. Total RNA was prepared from freshly isolated macrophages (lane 1), and from macrophages cultured either in the absence (lanes 2, 4, 6, and 8) or presence (lanes 3, 5, 7, and 9) of 100 U IL-4/ml. Each lane contains 5 μg of total RNA. RNA was electrophoresized, transferred onto a nylon membrane, hybridized with the ³²P-labeled CD13 cDNA probe, and exposed for 26 h (upper panel, A). The lower panel (B) shows the same blot rehybridized with the GAPDH cDNA probe, which was used as the internal standard for RNA content.

FIGURE 6. Kinetics of the IL-4-induced up-regulation of CD13 mRNA expression in human EC. Total RNA was prepared from EC cultured either in the absence of IL-4 (lanes 1, 3, 5, 7, and 9) or for 13 h, 25 h, 2, 3, and 4 days in the presence (lanes 2, 4, 6, 8, and 10) of 100 U IL-4/ml. Each lane contains 5 μg of total RNA. RNA was electrophoresized, transferred onto a nylon membrane, hybridized with the ³²P-labeled CD13 cDNA probe, and exposed for 3 days (upper panel, A). The lower panel (B) shows the same blot rehybridized with the GAPDH cDNA probe, which was used as the internal standard for RNA content.

increase was observed within minutes, indicating translocation of an intracellular pool of CD13 Ag to the cell surface. Together with our results presented here, this suggests that different mechanisms induce increased cell membrane expression of CD13 Ag. Rohrbach and Conrad reported that LAP activity in cultured PBM was enhanced by the presence of T lymphocytes (55). Although they did not speculate on the mechanism by which LAP 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. Kondepudi and Johnson studied another cell type and another cell membrane peptidase (56). They reported that several cytokines increased neutral endopeptidase activity in lung fibroblasts, and they suggested that up-regulation of this peptidase may limit inflammation in the pulmonary interstitium. In their study, several cytokines were shown to increase neutral endopeptidase activity in human lung fibroblasts, but our results show that IL-4 uniquely up-regulates CD13 Ag expression and aminopeptidase-N-activity in human monocytes and macrophages. Our conclusion is supported by others who have shown that IFN-γ and IL-10 were without effect on CD13 Ag expression in human monocytes (57).

Originally, the cell membrane glycoprotein (gp 150) defined by CD13 mAb has been regarded as a marker for subpopulations of hemopoietic cells (20-23). Nowadays, it is known to be expressed by a number of different cell types, including fibroblasts, the renal tubular and intestinal epithelium (24, 25), endothelial cells (26, 27), and synaptic membranes of cells of the central nervous system (28). Recently, the gene for this protein has been cloned, leading to the recognition of this protein as aminopeptidase-N (EC 3.4.11.2) (58). Aminopeptidase-N belongs to the group of ectoenzymes, so called because of their extracellularly located catalytic sites (25, 29, 30). It has been speculated that the actual physiologic substrate(s) of these ectoenzymes may
be either membrane bound, soluble, or part of the extracellular matrix. Another member of this group, neutral endopeptidase (EC 3.4.24.11, common acute lymphoblastic leukemia Ag, CD10), has been studied extensively. This enzyme has been reported to process a number of biologically active peptides (59), including IL-1β (60), substance P (29, 61), enkephalin (62), C5a (63), and FMLP (64). Less is known about the physiologic substrate(s) of aminopeptidase-N, but substrates comparable to those of neutral endopeptidase have been suggested (31). This is strengthened by the findings that different peptidases may be able to process the same peptide, or that inactivation of biologically active peptides may require sequential hydrolysis by a battery of distinct ectoenzymes in a multistep fashion (29, 30). In the central nervous system the role of aminopeptidase-N, together with other cell surface peptidases, is believed to be to inactivate neuropeptides (28). Comparatively, the action of peptidases at places of neurogenic inflammation (e.g., neurogenic inflammation in asthmatic lungs) is thought to be terminated, at least partially, by enzymatic degradation (65, 66). It has been reported that neutral endopeptidase is able to inactivate the neuropeptide substance P, which plays an important role in the pathogenesis of asthma (61). As hydrolysis of a particular peptide may result from the single action of different peptidases, and inactivation of biologically active peptides may need sequential hydrolysis by both endo- and exopeptidases (29, 30), aminopeptidase-N may, in concert with neutral endopeptidase, play a role in modulating inflammation in inflammatory diseases such as asthma.

As IL-4 has also been described in PBM as down-regulating cell membrane proteins such as CD14 Ag and the three Fcγ R (47), and the physiologic substrate of ectoenzymes may be membrane bound, IL-4-induced up-regulation of aminopeptidase-N may, in the same cell, play a decisive role in processing other cell membrane-bound Ag. Transfection experiments in CD14+ or Fcγ R+ cells using cDNA coding for enzymatically active aminopeptidase-N may clarify this issue.

The role of monocytes and macrophages in initiating and amplifying immunologic and inflammatory responses has been described extensively (67, 68). Both cell types may generate a variety of pro-inflammatory mediators and cytokines (68). On the other hand, it has been reported that macrophages may also be able to down-regulate inflammation (69). This down-regulation may be (partially) mediated by cell membrane peptidases. The IL-4-induced increase in LAP activity, as described here, may be a new, additional anti-inflammatory mechanism of IL-4.

In a role partly comparable to the role of monocytes and macrophages, EC are nowadays recognized as cells that actively participate in a variety of physiologic processes, such as inflammation and hemostasis (27, 70). Inflammation is characterized by perturbation of EC integrity, leading to increased vascular permeability. Several inflammatory peptides may be responsible for the increase in vascular permeability. A balanced regulation of production and degradation of these peptides determines the final development of localized edema. Recently, it was shown that neutral endopeptidase plays an important role in modulating the edema-forming effects of bradykinin (70). In view of the effects on aminopeptidase-N activity, IL-4 may therefore suppress indirectly the inflammatory response by increasing the capacity of EC to degrade pro-inflammatory peptides.

In conclusion, our results show that IL-4 is able to increase the expression of CD13 Ag on monocytes, macrophages, and endothelial cells. We postulate that up-regulation of CD13 Ag expression may be an indirect mechanism of IL-4 for modulating the action of bioactive peptides.

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