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Interleukin 4 promotes expression of mast cell ICAM-1 antigen

(cytokines/mast cells/recognition molecules)

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ABSTRACT **Cell recognition molecules play a crucial role in the regulation of immune cells. We recently found that mast cells (MCs) express leukocyte recognition molecules, including ICAM-1 antigen, a natural ligand of LFA-1. We here report that interleukin 4 (IL-4), a pleiotropic cytokine and mast cell differentiation factor, selectively promotes expression of surface ICAM-1 antigen and ICAM-1 mRNA in human MCs. IL-4 also up-regulates ICAM-1 antigen in cells of monocyte/macrophage lineage but has no effect on ICAM-1 antigen expressed on basophils, fibroblasts, or lymphocytes. The increase in expression of mast cell/macrophage ICAM-1 antigen induced by IL-4 may contribute to the accumulation of leukocytes and facilitate cell-contact-dependent regulation of immune cells in inflammed tissues.**

Mast cells (MCs) are primarily located in mucosal and perivascular areas of various tissues. They are known to play an important role in allergic events and immune reactions and may accumulate at sites of inflammation together with attracted leukocytes (1). Increasing evidence suggests that cell surface molecules play a significant role in the accumulation, distribution, and regulation of leukocytes in inflammatory reactions (2). We recently found that MCs express leukocyte recognition molecules such as Pgp-1 antigen (CD44) or ICAM-1 antigen (CD54) (3).

Interleukin 4 (IL-4) is a pleiotropic immunomodulator active on various cells of hemopoietic origin (4). Previous studies have shown that IL-4 is a differentiation factor for MCs (5, 6). We here provide evidence that IL-4, in addition, is involved in the regulation of MC ICAM-1 antigen.

MATERIALS AND METHODS

Cells. The human mast cell line HMC-1 was established from a patient suffering from MC leukemia (7). Other cell lines used are listed in Table 2. Primary cells were obtained from patients by standard techniques after informed consent was given. MCs were enriched from lung (bronchiogenic carcinoma, $n = 4$), juvenile foreskin ($n = 3$), ascitic fluid ($n = 4$), and gastrointestinal mucosa ($n = 2$). Lung MC ($n = 2$) and chronic granulocytic leukemia (CGL) basophils ($n = 2$) were highly purified (90–98% purity) by negative selection using monoclonal antibodies (mAbs) and complement (8). Mononuclear blood cells (MNCs) were obtained from two normal donors, from two chronic lymphocytic leukemia patients, and from two CGL patients. In addition, tonsillar B cells, cultured umbilical vein endothelial cells, and lung fibroblasts were analyzed. Cells were cultured in complete RPMI medium supplemented with 10% fetal calf serum in a humidified atmosphere of 5% CO_2 in air and 37°C.

Cytokines. Recombinant human IL-4 (rhIL-4) expressed in Chinese hamster ovary cells was purified by ion-exchange chromatography essentially as described by Widmer *et al.* (9). Purified rhIL-4 had a specific activity of 0.3×10^7 units/mg as defined by the amount of IL-4 required for half-maximal growth of T blasts precultured with 50 ng of rhIL-2 per ml and 1 μg of phytohemagglutinin per ml for 5 days. rhIL-2, rhIL-3, rhIL-8, and rh-granulocyte/macrophage-colony-stimulating factor (rhGM-CSF) were provided by Sandoz. rhIL-1, rhIL-6, and rh-macrophage-CSF were purchased from Genzyme, rh-interferon α and interferon γ were from Boehringer Ingelheim, and rh-granulocyte-CSF was from Amersham.

Stimulation with Cytokines. Cells were incubated with rhIL-4 (1, 10, or 100 ng/ml) or control medium for 4 days unless otherwise stated. In some experiments, rhIL-4 was preincubated with either control medium or mAb 1-398'18 specific for IL-4. mAb 1.398'18 (IgG1 subclass) was produced by immunizing BALB/c mice (with rhIL-4) essentially as described by Köhler and Milstein (10). HMC-1 cells were also exposed to other cytokines (see above). After various periods of time cells were analyzed for histamine content, surface marker expression, mRNA expression, and uptake of thymidine.

mAbs and Immunofluorescence Analysis. For surface membrane analyses, cells were incubated with mAbs, stained by indirect immunofluorescence, and analyzed by flow cytometry (FACS 440, Becton Dickinson) as reported (3, 8). mAbs used are listed in Table 2. Blood monocytes and lymphocytes were analyzed in MNC samples as gated populations. Tissue MCs were analyzed by combined toluidine blue/immunofluorescence staining (3, 8).

mRNA Analysis. Total RNA was extracted from cells by the guanidinium isothiocyanate/cesium chloride method described by Chirgwin *et al.* (11). In brief, 10 μg of RNA was size-fractionated on 1.2% agarose gels and transferred to synthetic membranes (Hybond N, Amersham) with 20× SSC (1 × SSC = 150 mM NaCl/15 mM sodium citrate, pH 7.0) overnight. Filters were baked at 80°C for 2 hr and then prehybridized at 65°C for 4 hr in 5× SSC, 10× Denhardt's solution (DhS) (1× DhS = 0.02% bovine serum albumin/0.02% polyvinylpyrrolidone/0.02% Ficoll), 10% dextran sulfate, 20 mM sodium phosphate (pH 7.0), 7% SDS, sonicated salmon sperm DNA (100 μg/ml), and poly(A) (100 μg/ml). Hybridization was performed with a ^{32}P-labeled, synthetic, 30-mer ICAM-1 oligonucleotide probe [gene position, 360–370; Simmons *et al.* (12)] for 16 hr at 65°C in the prehybridization buffer. The probe was labeled by terminal nucleotidyltransferase and [α-^{32}P]dATP. Blots were washed once in 5% SDS, 3× SSC, 10× DhS, and 20 mM sodium phosphate (pH 7.0) for 30 min at 65°C and once in 1× SSC/1% SDS for

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Abbreviations: mAb, monoclonal antibody; IL, interleukin; CGL, chronic granulocytic leukemia; rh, recombinant human; MC, mast cell; MNC, mononuclear blood cell.

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30 min at 65°C. Bound radioactivity was visualized by exposure to XAR-5 films at −70°C using intensifying screens (Kodak).

Proliferation Assay. As a marker of proliferation, [^{3}H]-thymidine uptake was quantified. HMC-1 cells (10^5 per well) were incubated with rhIL-4 (1, 10, and 100 ng/ml), a mixture of rhIL-4 and mAb 1.398'18 (anti-IL-4), or control medium in the wells of 96-well microtiter plates (Costar). On days 0, 2, 5, and 9, 1 μCi of [^{3}H]thymidine (1 Ci = 37 GBq; New England Nuclear) was added. Twelve hours later, incorporated radioactivity was harvested on glass fiber filters and assayed in a liquid scintillation counter.

Histamine Measurement. Cellular histamine was measured by radioimmunoassay (Immunotech, Marseille, France) as described (8) after cell lysis in distilled water and freeze-thawing.

Receptor Assay. rhIL-4 was radiolabeled by the Pierce Iodo-Gen procedure. In brief, 5-μg samples of rhIL-4 were incubated with 10 μg of Iodo-Gen and 0.5 mCi of $Na^{125}I$ in 50 μl of 0.2 M phosphate buffer (pH 7.2) at 20°C for 15 min, and then the incubation mixture was loaded on a Pharmacia PD 10 G-25 M column equilibrated with phosphate-buffered saline/0.25% gelatin. IL-4 was separated from nonincorporated iodine by gel filtration in the same buffer. Specific radioactivity of iodinated rhIL-4 was measured by self-displacement analysis as described by Calvo *et al.* (13). For binding studies, 2×10^6 cells were incubated with various concentrations of ^{125}I-labeled rhIL-4 in 100 μl of RPMI 1640 medium containing 20 mM Hepes buffer, 10% fetal calf serum, and 0.2% sodium azide at pH 7.2 (binding medium). After 2 hr of incubation at 4°C in 1.5-ml Eppendorf tubes, the incubation mixture was transferred to 400-μl Beckman tubes containing 200 μl of precooled fetal calf serum. Cells were centrifuged for 2 min (Eppendorf centrifuge model 5413). Medium and serum were then removed by aspiration and the radioactivity of the cell sediment was assayed in a γ counter. Nonspecific binding of IL-4 was quantified in the presence of 20- to 100-fold excess of unlabeled rhIL-4. Numbers of binding sites and constants were calculated using the LIGAND program (G. A. McPherson, Biosoft Cambridge, Cambridge, MA).

Statistical Analyses. The significance of differences was assessed using standard statistical tests including Colmogorov–Smirnov analysis for the FACS data. The results were considered significantly different when $P < 0.05$.

Table 1. Leukocyte adhesion molecules expressed on human MCs

CD*	Antigen	Natural ligand	mAb	Score reactivity† HMC-1	lu-mc	a-mc	s-mc	g-mc
02	LFA-3 R	LFA-3	T11	3	1	1	1	1
02	LFA-3 R	LFA-3	VIT-13	3	1	NT	1	NT
04	T4	DR II	Leu-3	1	1	1	1	1
08	T8	DR I	Leu-2	1	1	1	1	1
11a	LFA-1	ICAM	MHM24	1	1	1	1	1
18	LFA-1β	ICAM	MHM23	1	1	1	1	1
44	Pgp-1	?	F10-44-2	4	4	NT	4	NT
54	ICAM-1	LFA-1	LB-2	3	1	NT	1	NT
54	ICAM-1	LFA-1	R.I.1.1.1.	3	1	NT	NT	NT
54	ICAM-1	LFA-1	84H10	3	2	2	2	2
54	ICAM-1	LFA-1	My-13	3	2	NT	2	NT
54	ICAM-1	LFA-1	8F5	3	1/2	2	1/2	2
58	LFA-3	CD2	TS2/9	3	NT	NT	NT	NT

Primary MCs were isolated using collagenase and examined for reactivity with mAbs by toluidine blue/immunofluorescence staining and/or flow cytometry as described (3, 8). HMC-1 cells were analyzed by flow cytometry. mAbs were obtained from the International Workshop and Conference on Human Leukocyte Differentiation Antigens (14).

*CD refers to the International Workshop and Conference on Human Leukocyte Differentiation Antigens (Fourth Workshop, ref. 14). lu-mc, Lung MCs; a-mc, ascites MCs; s-mc, skin MCs; g-mc, gastrointestinal MCs; NT, not tested.

†Score: 1 = <20% of cells reactive; 2 = 20–40% of cells reactive; 3 = 40–60% of cells reactive; 4 = 60–80% of cells reactive; 5 = 80–100% of cells reactive.

RESULTS

Expression of Leukocyte Recognition Molecules on Human MCs. MCs enriched from lung, ascitic fluid, skin, or intestinal mucosa as well as HMC-1 cells were recognized by CD54 mAb 84H10. Human MCs were also stained by other CD54 mAbs, although the intensity of staining was low or at the detectable limit (Table 1). HMC-1 cells apparently express LFA-3 (CD58) and CD2 (LFA-3 receptor) antigen. Primary MCs and HMC-1 cells express Pgp-1 antigen (CD44) but lack LFA-1 (CD11/CD18) molecules (see Table 1).

Effect of rhIL-4 on MC ICAM-1 Antigen. Unstimulated MCs express low levels of ICAM-1 antigen. rhIL-4 (but not other lymphokines) induced an increase in expression of ICAM-1 antigen on HMC-1 in six of six experiments performed ($P < 0.001$; see also Fig. 1). IL-4 also induced an increase of ICAM-1 antigen on primary lung MCs and cells of monocyte/macrophage lineage—i.e., blood monocytes, U937, THP-1, and Mono-Mac-1—after 4 days of culture (Table 2). In contrast, rhIL-4 failed to promote expression of ICAM-1 antigen on CGL basophils, KU812 (a basophil cell line), endothelial cells, fibroblasts, or cells of lymphocyte lineage (Table 2). rhIL-4 failed to

Table 2. IL-4-induced up-regulation of ICAM-1 antigen on human target cells

Cell line	Cell type	Constitutive expression	IL-4-induced up-regulation of ICAM-1: Cell surface	mRNA
HMC-1	Mast cell	+	+	+
KU812-F	Basophil precursor	+	−	NT
HL-60	Myeloid	+	−	NT
KG-1a	Myeloid	+	−	−
HEL	Myeloid	+	−	NT
U937	Monocytic	+	±	−
Mono-Mac-1	Monocytic	+	+	±
Mono-Mac-6	Monocytic	+	−	−
THP-1	Monocytic	+	+	+
Raji	B cell	+	−	−
Daudi	B cell	+	−	NT
Nalm-6	B cell	+	−	−
REH-6	B cell	+	−	NT
HUT-104	T cell	+	−	NT
Molt-4	T cell	+	−	−
CCD-MLu	Lung fibroblast	+	−	NT
HeLa	Carcinoma	+	−	−
—	Purified lung MC	±	+	NT
—	Pure CGL basophil	+	−	NT
—	Monocyte (gated)	+	+	NT
—	Blood lymphocyte	+	−	NT
—	Tonsillar B cell	+	−	NT
—	CGL (MNC)	+	−	NT
—	CLL (MNC)	+	−	NT
—	Lung fibroblast	+	−	NT
—	HUVEC	+	−	−

Cells were stained for surface ICAM-1 antigen and probed for ICAM-1 mRNA. For uninduced cells, + means expression of ICAM-1 antigen, and for induced cells, + means significant up-regulation of ICAM-1 antigen by IL-4. CGL, chronic granulocytic leukemia; HUVEC, human umbilical vein endothelial cell.

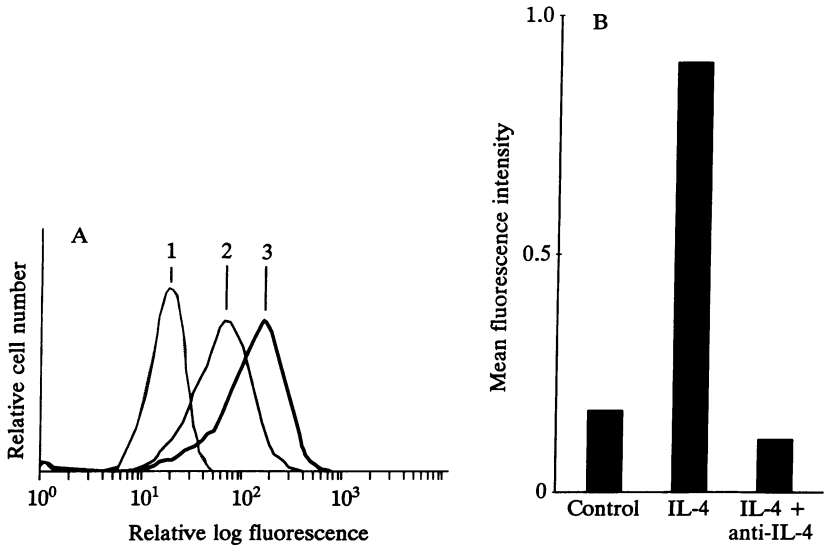

FIG. 1. Up-regulation of ICAM-1 antigen by rhIL-4 on HMC-1. (*A*) Flow cytometry of HMC-1 using mAb LB-2 (anti-ICAM-1). No. 1 indicates staining with an irrelevant control antibody of IgG1 subclass after preincubation with control medium (—) or rhIL-4 (·····). No. 2 indicates staining of HMC-1 with mAb LB-2 after incubation in control medium for 4 days. No. 3 indicates staining with LB-2 after induction with rhIL-4 (100 ng/ml) for 4 days. (*B*) Inhibition of the IL-4 effect by mAb specific for IL-4. rhIL-4 (100 ng/ml) was incubated with mAb 1.398'18 (50 μg/ml) or control medium for 3 hr. Then, cells were incubated with IL-4 plus medium versus IL-4 plus anti-IL-4 for 4 days. Afterwards, HMC-1 cells were washed and incubated with mAb LB-2 (CD54) for 30 min and stained with sheep anti-mouse $F(ab)_2$ IgG plus IgM and analyzed by flow cytometry. Bars indicate mean fluorescence intensity (MFI) of HMC-1 after subtraction of nonspecific MFI obtained with a control mAb of IgG1 subtype.

promote expression of other adhesion or differentiation antigens (CD2, CD9, CD11/18, CD33, CD44, YB5B8) (not shown) on HMC-1. Levels of surface ICAM-1 antigen on HMC-1 induced by IL-4 (10 or 100 ng/ml) reached maximum levels by day 4 and thereafter decreased to baseline levels between days 9 and 14. The effect of rhIL-4 on expression of ICAM-1 was dose dependent (optimal concentration: 10 ng/ml or above) and could completely be neutralized by mAb 1.398'18 specific for IL-4 (Fig. 1*B*).

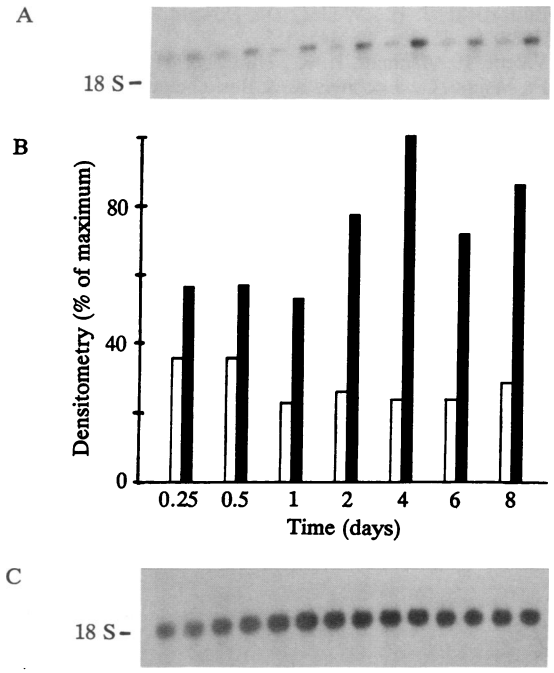

FIG. 2. Up-regulation of ICAM-1 mRNA in MCs by rhIL-4. (*A*) Northern blot analysis of HMC-1 cultured in control medium (open bars) or rhIL-4 (100 ng/ml; filled bars) for 8 days using an ICAM-1 oligonucleotide. (*B*) Relative density of signals obtained by laser densitometry. (*C*) β-Actin mRNA from the same blot.

Using an ICAM-1-specific oligonucleotide probe we were able to show that IL-4 promotes expression of ICAM-1 mRNA in HMC-1 (in four of four experiments performed) and in cells of monocyte/macrophage lineage (Fig. 2, Table 2). This increase in ICAM-1 mRNA (4.1 ± 1.3-fold for HMC-1 and about 2- to 3-fold for THP-1, as measured by densitometry) corresponds with the IL-4-dependent increase in surface ICAM-1 antigen (i.e., 3- to 10-fold increase for HMC-1 in FACS profiles).

Effect of IL-4 on [^{3}H]Thymidine Uptake and Synthesis of Histamine in HMC-1 Cells. rhIL-4 was found to down-regulate uptake of [^{3}H]thymidine in HMC-1 cells in a dose-dependent manner ($P < 0.005$) (see Table 3). IL-4 also induced a decrease in total cell number and a marginal increase in cellular histamine compared with baseline histamine levels (baseline level: 100–150 ng per 10^5 cells).

Binding of Iodinated rhIL-4 to Human MCs. To demonstrate IL-4 binding sites on HMC-1, cells were incubated with increasing concentrations of iodinated rhIL-4. Scatchard transformation of the IL-4 binding sites calculated by the LIGAND program is shown in Fig. 3. IL-4 specifically binds to

Table 3. IL-4-induced inhibition of [^{3}H]thymidine uptake by HMC-1 cells

Stimulus	cpm × 10^{-4} on day 5 of culture		
	Exp. 1	Exp. 2	Exp. 3
Control medium	36.16 ± 3.42	28.06 ± 0.51	21.73 ± 1.01
IL-4, ng/ml			
1	23.74 ± 3.38	24.35 ± 1.53	18.87 ± 3.28
10	16.61 ± 2.65	22.40 ± 1.19	09.20 ± 0.41
100	15.85 ± 3.48	15.05 ± 1.55	08.80 ± 1.02
200	17.15 ± 1.11	15.24 ± 2.86	NT
100 + anti-IL-4	31.75 ± 0.93	NT	NT

HMC-1 cells were induced with rhIL-4 preincubated with either control medium or anti-IL-4 mAb 1.398'18. After incubation, [^{3}H]thymidine was added and 12 hr later, incorporated radioactivity was harvested on glass fiber filters and assayed in a liquid scintillation counter. Three independent experiments are depicted. Results represent the mean ± SD of triplicate determinations. NT, not tested.

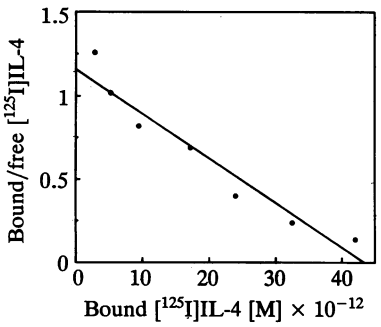

FIG. 3. Expression of the IL-4 receptor on HMC-1: Scatchard transformation of IL-4 binding sites from one representative experiment.

a single class of 1.100–2.400 high-affinity binding sites on HMC-1 cells with a calculated dissociation constant, K_{da}, of 0.04–0.2 nM (ranges from four independent experiments).

DISCUSSION

ICAM-1 antigen, a multifunctional recognition molecule, is expressed on various mesenchymal cells. Previous studies have shown that expression of ICAM-1 antigen on fibroblasts, endothelial cells, or lymphocytes is regulated by cytokines (2). More recently, we have shown that MCs express the ICAM-1 antigen. In the present study we provide evidence that IL-4 selectively promotes expression of MC/macrophage ICAM-1 antigen.

HMC-1 is a newly established human MC line (7). Using this cell line we were able to partially characterize the mechanism of IL-4-induced up-regulation of MC ICAM-1 antigen. IL-4 obviously stimulates HMC-1 cells through high-affinity binding sites. This confirms earlier studies performed with murine MC lines (15). The observation that IL-4 promotes expression of ICAM-1 mRNA in HMC-1 cells may indicate that this cytokine directly regulates expression of the ICAM-1 gene. Alternatively, this change in mRNA expression is due to posttranscriptional regulation.

The effect of IL-4 on MC ICAM-1 antigen appears to be specific in many respects. IL-4 had no influence on other recognition molecules expressed on MCs. IL-4 also had no effect on cells other than MCs or mononuclear phagocytes, which probably share a common differentiation pathway (8). Furthermore, of several lymphokines tested, only IL-4 was found to promote expression of ICAM-1 antigen in MCs.

Many leukocytes express LFA-1, the principal ligand of ICAM-1. During inflammatory processes these cells migrate into tissues upon chemoattractive stimulation. The IL-4-induced increase in expression of the ICAM-1 antigen on tissue MCs and tissue macrophages may play a role in the accumulation, distribution, and regulation of leukocytes in inflamed tissues.

Previous studies have shown that IL-4 regulates differentiation of murine MCs (5, 6). A differentiation factor for human MCs has so far not been characterized. In this study IL-4 induced only a marginal increase in the per cell histamine content of HMC-1 cells and IL-4 also failed to induce and/or promote other MC differentiation antigens (such as YB5B8) on human MCs. More recent data suggest that optimal growth of human MCs (progenitors) depends on the presence of stroma cells (16). Such stroma cells may interact with MCs through recognition molecules such as ICAM-1. Whether the ICAM-1 antigen *per se* plays a role in the differentiation, commitment, or tissue location of MCs, remains to be elucidated. It also remains unknown whether all MCs express ICAM-1 antigen on their surface or not. In particular, in the combined toluidine blue/immunofluorescence staining experiments the intensity of staining was low and for some CD54 mAbs even at or below the detectable limit. However, this phenomenon may well be due to the low number of ICAM-1 molecules expressed on tissue MCs. IL-4 is a multipotent lymphokine involved in the regulation of immune responses (4). In particular, IL-4 has been shown to induce not only differentiation of murine MCs but also growth and function of lymphocytes and synthesis of IgE. The observation that IL-4 up-regulates ICAM-1 antigen on macrophages and MCs further supports the concept that IL-4 is a central mediator in immunregulatory interactions of cells relevant to allergic and/or inflammatory processes.

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