Interleukin-4 (IL-4) and IL-13 Bind to a Shared Heterodimeric Complex on Endothelial Cells Mediating Vascular Cell Adhesion Molecule-1 Induction in the Absence of the Common γ Chain

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Interleukin-4 (IL-4) and IL-13 exert similar, nonadditive effects on endothelial cells, inducing vascular cell adhesion molecule-1 (VCAM-1) expression and subsequent transmigration of eosinophils. The receptor for IL-4 and IL-13 was described as a shared heteromultimeric complex in which the common γ-chain (γc) subunit was essential for activity. Endothelial cells bound both cytokotins with high affinity; by flow cytometry and reverse transcription-polymerase chain reaction (RT-PCR), they expressed IL-4 receptor α (IL-4Ra) but did not express the γc of the IL-2R. Radioligand cross-linking experiments followed by immunoprecipitation with the monoclonal antibody (MoAb) S697 to the IL-4γc, in turn, is a specific ligand of VLA-4γ that has a distinct function in the orchestrated cascade of molecular interactions governing penetration of the leukocytes across the endothelial barrier.

The IL-4 receptor (IL-4R) is a 130-kD transmembrane protein consisting of a 220 amino acid extracellular domain including two pairs of cystein residues and the typical WSXWS motif of the hematopoietin or type-I cytokine receptor superfamily. Its unique large intracellular domain of 553 amino acids was found to be essential for most IL-4-specific functions.

In addition, IL-2R, IL-4R, IL-7R, IL-9R, and IL-15R share a common subunit referred to as γc.

The IL-13R has been proposed as an additional candidate using the γc as part of the receptor complex. There is convincing evidence that γc is not binding IL-4 by itself, although it associates with the 130-kD IL-4R and enhances its affinity with the ligand.

IL-4 and IL-13 have remarkably similar functions on different leukocytic cells. The observation that different cell lines, although expressing distinct surface receptors for IL-4 and IL-13, share common receptor subunits supports these data. Sharing of γc on most cells adds additional complexity to the understanding of the IL-4/IL-13R biology and raises the question whether the γc is required for signal transduction.

The IL-13R is expressed on many cells, including endothelial cells, and is of the mouse IgG class.

MATERIALS AND METHODS

Reagents.

The Chinese hamster ovary-derived human IL-13 and a mutant form of human IL-4, IL-4 Y 124D, were kindly provided by Dr G. Zurawski (DNAX Research Institute of Cellular and Molecular Biology, Palo Alto, CA). We acknowledge the generous supply of human IL-1 by Dr P.T. Lomedico (Hoffmann-La Roche, Nutley, NJ) and of tumor necrosis factor (TNF) by Dr Z. Nagy (Preclinical Research, Sandoz Ltd, Basel, Switzerland). Purified human IL-4 (107 U/mg) produced in Chinese hamster ovary cells and the monoclonal antibodies (MoAbs) S697 and S103 were a gift of Dr J. Banchereau (Schering Plough, Dardilly, France). The antibodies are of the mouse IgG class and were raised against the extracellular domain of the human IL-4γc. The MoAb S299-27-1 directed against VCAM-1 is of the mouse IgG2a class and was purchased from BMA Biomedicals AG (Augst, Switzerland). The phycoerythrin-labeled polyclonal antibody was from Sigma (St Louis, MO). TUGh4 is a rat MoAb of the IgG2a class, raised against the rat transfectant cell line TARTH γc-15 expressing the human γc. The MoAb was thoroughly characterized by immunoprecipitation using MOLT-β cells expressing γc and
the IL-2Rβ chain. In these experiments the MoAb specifically precipitated a protein with a molecular mass of 65 to 70 kD, which corresponds to that of the γc.21

Genistein, staurosporine, H7, herbinycin A, typhostin A46 and A47, wortmannin, and okadaic acid (Calbiochem, San Diego, CA) were either diluted in medium or in dimethyl sulfoxide (DMSO) and used at indicated concentrations using final concentrations of DMSO at 1:1000 (vol/vol). Sodium-orthovanadate was purchased from Sigma, and disuccinimidyl suberate was from Pierce (Rockford, IL).

**Cell cultures.** Human umbilical vein endothelial cells (HUVECs) were harvested as previously described.23 The cells were seeded on purified human fibronectin (Winger AG, Wohlen, Switzerland) and grown in Medium 199 enriched with sodium heparin (90 μg/mL; Novo Industries, Copenhagen, Denmark) and endothelial cell growth supplement (15 μg/mL; Collaborative Research, Inc, Waltham, MA) in the presence of 20% pooled human serum. Final monolayers were used in their second to fourth passage showing 90% confluent HUVEC monolayers were activated with IL-4 and IL-13 for 16 hours before transendothelial migration was determined in a 120-minute assay. (A) Bilayer vascular constructs were exposed to medium containing 5 nmol/L either of IL-4 (■), IL-13 (□), or both together (hatched bars). Control bilayers (cross-hatched bars) were incubated in medium alone. (B) Bilayers were pretreated with IL-4 (●) or IL-13 (○) at indicated concentrations. Data points are means ± SD of a representative experiment.

**Fig 1.** Induction of transendothelial migration of eosinophils by IL-4 and IL-13. Bilayer vascular constructs consisting of fibroblast-derived extracellular matrix covered by confluent HUVEC monolayers were activated with IL-4 and IL-13 for 16 hours before transendothelial migration was determined in a 120-minute assay. (A) Bilayer vascular constructs were exposed to medium containing 5 nmol/L either of IL-4 (■), IL-13 (□), or both together (hatched bars). Control bilayers (cross-hatched bars) were incubated in medium alone. (B) Bilayers were pretreated with IL-4 (●) or IL-13 (○) at indicated concentrations. Data points are means ± SD of a representative experiment.
of eosinophils per high power field (0.25 mm²) is given as the percentage of migrated eosinophils.

*Flow cytometric analysis.* Adherent cells were grown to confluence in 6-well dishes (Falcon; Becton Dickinson Labware, Franklin Lakes, NJ). Stimuli were added directly to the medium 16 hours before the cells were washed once in 20 mmol/L PBS; detached shortly (1 minute) in 0.05% trypsin/0.02% EDTA/PBS, pH 7.4; blocked with FCS; and suspended in PBS containing 1% bovine serum albumin (BSA; wt/vol; PBS-A). A total of 5 × 10⁵ cells were incubated with the indicated MoAbs at 10 µg/mL for 45 minutes at 4°C and were subsequently washed twice in cold PBS-A. In control experiments, we used an irrelevant isotype-matched control MoAb (Caltag, San Francisco, CA). Goat antimouse IgG-phycocerythrin R Ab, diluted 1:30 (Sigma), was then applied for 30 minutes; the cells were washed twice and resuspended in PBS-A at 4°C. Flow cytometry was performed within 12 hours using a Becton Dickinson flow cytofluorograph (FACScan A2; Becton Dickinson & Co, Sunnyvale, CA). Fluorescence intensity was determined from 10,000 cells per sample using the LYSIS II software (Becton Dickinson).

Reverse transcription (RT) and polymerase chain reaction (PCR). Total cellular RNA was isolated by the guanidine isothiocyanate method²⁶ and quantified at 260 nm. Total RNA (20 µg) was reverse-transcribed in a reaction volume of 30 µL. One microliter of this reaction was then amplified by PCR. Briefly, RNA was mixed with oligo-(dT)₁₀₋₂₀ (200 ng), heated for 5 minutes at 70°C, and quick-chilled in an ice bath. A total of 200 U of moloney murine leukemia virus-reverse transcriptase (GIBCO-BRL) in 50 mmol/L Tris-HCl (pH, 8.3), 75 mmol/L KCl, 3 mmol/L MgCl₂, 0.2 mmol/L of each deoxynucleotide triphosphate, and RNase inhibitor (50 U) were added to a final volume of 30 µL. RT was performed at 37°C for 2 hours. The specific primer pairs for the

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**Fig 2.** Induction of VCAM-1 expression on HUVECs. HUVEC monolayers were incubated in medium containing 0.1 nmol/L of the indicated cytokines. Control monolayers (Resting HUVEC) were kept in medium alone. The mean fluorescence intensity (MFI) and the immunopositive population (given in percent [%]) within the margins (M1) are indicated. The results are from one representative experiment using the same batch of endothelial cells.
human IL-4Ra, γc, and the human and mouse β2-microglobulin were custom-synthesized and quality-checked by capillary electrophoresis by MWG-Biotech (Ebersberg, Germany). The human and mouse β2-microglobulin primers were as described. The human sense primer was 5'-CCAGCAAGAATGGAAAGTC-3', and the antisense primer was 5'-TGACCAGGCTTGTATGCTATC-3' spanning a 222-bp fragment; mouse sense primer was 5'-CAGTGTGAAGAT-3' spanning a 268-bp fragment; mouse sense primer was 5'-TGACCGGCT-TGTATGCTATC-3' and antisense primer was 5'-CAGTGTGAAGAT-3' spanning a 1448-bp fragment; mouse sense primer was 5'-TGACCGGCT-TGTATGCTATC-3' and antisense primer was 5'-CAGTGTGAAGAT-3' spanning a 222-bp fragment. The human IL4-Ra sense primer was 5'-GGAGAGGGTATAGGCCTT-3', and the antisense primer was 5'-GATGTCGGTTACGTGCG-3' spanning a 571-bp fragment; the γc sense primer was 5'-GAGACAAGCGGCATGGTGA-3', and the antisense primer was 5'-GATGATTACAGAAGACTT-3' spanning a 1488-bp fragment. PCR reactions were performed in a final volume of 25 μL in 500-μL microtubes (Perkin Elmer-Cetus, Norwalk, CT), and each sample was overlaid with 25 μL paraffin oil. The mixtures contained 1 μL of RT-reaction in 10 mmol/L Tris-HCl (pH, 9.0; IL-4Ra and β2-microglobulin), 50 mmol/L Tris-HCl (pH, 9.0; γc), 50 mmol/L KCl, 1.6 mmol/L MgCl2, (IL-4Ra), 1.0 mmol/L MgCl2 (γc), 0.8 mmol/L MgCl2 (β2-microglobulin), 0.01% gelatine, 0.1% Triton X-100, 0.2 mmol/L of each deoxynucleotide triphosphate, 0.8 μmol/L of each primer, and 0.5 U Super Taq polymerase (P.H. Stehelin & Cie AG, Basel, Switzerland). Human γc was amplified using 35 cycles at 94°C (for 1 minute), at 55°C (for 1 minute), and at 72°C (for 1 minute) in a thermal cycler (Perkin Elmer-Cetus). β2-Microglobulin and IL-4Ra samples were amplified using 35 cycles at 94°C (for 30 seconds) and at 53°C (for 30 seconds). Twenty microliters of each RT-PCR reaction were run on a 2% (IL-4Ra and β2-microglobulin) or 1% (γc) agarose gel containing 0.2 μg/mL ethidium bromide in 1× Tris-acetate/EDTA electrophoresis buffer. DNA digested with EcoRI and HindIII (Boehringer Mannheim, Germany) and pUC 12 DNA digested with Hind II were used as DNA molecular weight markers.

**Iodination of IL-4 and IL-13.** Iodination of IL-4 (2.5 μg) or IL-13 (2.5 μg) was performed using 5 μg Iodogen (Pierce) in the presence of 1 mCi Na125I (Amersham International, Bucks, UK); free 125I was removed by chromatography on a PD-10 column (Pharmacia) equilibrated with 20 mmol/L PBS (pH, 7.4) containing 1% (wt/vol) BSA. The specific activities of [125I]IL-4 and [125I]IL-13 were 60 to 70 μCl/mg and 90 to 100 μCi/mg, respectively. Both cytokines had no detectable loss of biological activity. The radiolabeled cytokines were stored in PBS-A at -20°C.

**Binding of [125I]IL-4 and [125I]IL-13 to endothelial cells.** Differentiated HUVECs and ECV304 cells were shortly trypsinized, washed with ice-cold PBS, resuspended in DMEM containing 1% BSA, and kept on ice. A total of 2×10⁶ cells and various concentrations of [125I]IL-4 or [125I]IL-13 were mixed in a total volume of 200 μL. The mixture was incubated for 1 hour at 4°C. At the end of the incubation, the suspension was layered onto silicon oil, centrifuged at 8,000 g for 1.5 minutes, and immediately frozen on dry ice, and the tubes were cut at the pellet. Bound radioactivity of the sedimented cells and the residual label in the supernatant were counted. Nonspecific binding was measured by incubating the same number of cells with an excess of 1 μmol/L unlabeled IL-4 and 0.23 μmol/L of IL-13 at 4°C for 20 minutes. Binding data were analyzed with the computerized weighted least-square curve-fitting software described by Munson and Rodbard.

**Affinity cross-linking.** Adherent cells were trypsinized as described above. The detached cells were washed 3 times in PBS and resuspended in DMEM-medium containing 1% BSA. Aliquots of 200 μL containing 4×10⁶ cells were incubated with [125I]IL-4 (0.5 nmol/L) or [125I]IL-13 (3 nmol/L) on ice for 1 hour. For competition, an excess of 1 μmol/L unlabeled ligand was added 20 minutes before the iodinated cytokines were added. The cell suspension was layered carefully onto 3 mL of 10% sucrose/PBS, kept at 4°C, and centrifuged at 18,000 g for 2 minutes. The pellet was dissolved in 250 μL PBS, and cross-linking was performed for 15 minutes at room temperature (RT) by adding 5 μL disuccinimidyl suberate (10 mg/mL DMF; Pierce). The reaction was stopped with 750 μL of Tris-buffered saline for 5 minutes at room temperature and was centrifuged at 10,000 g for 2 minutes at 4°C. The cell pellet was solubilized for 40 minutes on ice in 50 μL lysis buffer containing 2% (vol/vol) NP-40, 0.1% (wt/vol) EDTA, and 1 μg/mL aprotinin, leupeptin, pepstatin, and phenylmethyl sulfonyl fluoride. After centrifugation for 15 minutes at 10,000 g, the supernatant was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions using 3% to 10% (wt/vol) polyacrylamide gels. Autoradiography was performed at -70°C exposing x-ray Hyperfilms (Amersham International) for 2 to 3 weeks.

For immunoprecipitation, the cell lysates were cleared with denatured pansorbin (Calbiochem, San Diego, CA) that had been labeled with control IgG for 30 minutes. After incubation with specific antibody-labeled pansorbin for 14 hours, the precipitates were
RESULTS

Biological effects of IL-4 and IL-13 on endothelial cells. IL-4 and IL-13 have similar effects on HUVECs in culture, augmenting the adherence of VLA-4-expressing cells. IL-4-induced adherence resulted in subsequent transendothelial migration of eosinophils through the bilayer vascular construct. We show that IL-13 evokes transendothelial migration of eosinophils similarly to IL-4 and that IL-4 and IL-13 exert no additive effects (Fig 1). Similar nonadditive effects were obtained at different suboptimal concentrations of IL-4 and IL-13 (data not shown). However, HUVECs were more sensitive to IL-4 than to IL-13. Half-maximal transendothelial migration was observed at 0.06 ± 0.01 nmol/L IL-4 and at 0.9 ± 0.3 nmol/L for IL-13 (n ± SEM of three experiments).

Flow cytometric analysis of IL-4-activated HUVEC monolayers showed significant induction of VCAM-1, with up to 65% immunopositive cells (Fig 2). The responding population expressing detectable VCAM-1 was variable among the different HUVEC batches. Such variability was recently reported. VCAM-1 induction peaked at 16 hours and lasted for at least 72 hours of IL-4 stimulation (data not shown). When HUVECs were exposed to IL-13, VCAM-1 expression was essentially the same, and the population of immunopositive HUVECs was comparable (Fig 2). The time dependence obtained with IL-13 was identical to that of IL-4 (data not shown). Preincubation of the monolayers with both cytokines neither enhanced the level of VCAM-1 expression nor enlarged the population of VCAM-1-positive cells. However, clear cooperation was observed when either IL-4 or IL-13 was combined with TNF (Fig 2). Similar results were obtained with IL-1 instead of TNF (data not shown).

Mutant IL-4 substituted at Tyr124 for Asp (IL-4Y124D), which is known to bind to the IL-4Rα, did not induce VCAM-1 and, when preincubated in a 100-fold excess, competitively antagonized VCAM-1 induction by native IL-4 and IL-13 (Fig 3). These data indicate a shared signaling subunit in the IL-4 and IL-13 receptor complex.

Flow cytometric analysis of the cloned IL-4R subunits on endothelial cells and various cell lines. The composition of the IL-4R complex was studied in HUVECs, adult saphenous vein endothelial cells, and a variety of cell lines. We examined IL-4Rα with the nonblocking MoAb S697 that was raised to a 200 amino acid extracellular portion of the human IL-4Rα chain. Figure 4 shows that HUVECs clearly ex-
press the IL-4Ra, though at a low density (8.4 ± 1.2 specific fluorescence units; n = SEM of five experiments). Similar expression of IL-4Ra was detected on adult saphenous vein endothelial cells (data not shown), MLA-144; a gibbon T-cell line; and HMC-1, a human mast cell line (Fig 4). B16, a mouse fibroblast cell line stably transfected with the human IL-4Ra cDNA, was used as a positive control.

The γc of the IL-2R has been thought to be an obligate component of the IL-4R, forming a heteromultimeric complex including the IL-4Ra chain.17 We studied the expression of γc on the above listed cell types using TUGh4, a rat MoAb directed against recombinant human γc.22 Both cell lines, MLA-144 and HMC-1, were clearly γ-chain-positive. However, HUVECs (Fig 4) and adult saphenous vein endothelial cells (data not shown) did not express detectable levels of γc.

Receptor-binding analysis of radiolabeled IL-4 and IL-13 to the HUVEC line ECV304. Binding of radiolabeled IL-4 and IL-13 to HUVECs and analysis of the data by the weighted least-square curve-fitting method29 showed one class of binding sites for both cytokines (Fig 5). These data showed for IL-4 a kd value of 33.0 ± 13.0 pmol/L and 228 ± 82 receptors per cell. IL-13 bound with a kd value of 63.0 ± 16.0 pmol/L to 700 ± 175 receptors per cell. Similar data were obtained with the endothelial cell line ECV304 (data not shown).

Detection of specific mRNA for IL-4Ra- and γc-chain. Expression of IL-4Ra- and γc-specific mRNA was studied by the RT-PCR method. Using appropriate primers (see Materials and Methods) a single 557-bp fragment encoding the 55-kD component of the IL4R, forming an heteromultimeric complex on HWECs.

Radioligand cross-linking of the IL-4 and IL-13 receptor complex on HUVECs. To investigate the subunit structure of the endothelial IL-4/IL-13 receptor complex iodinated IL-4 or IL-13 was cross-linked by the disuccinimidyl suberate method to its functional binding sites on the cell surface. The interacting subunits were analyzed by SDS-PAGE calculating the net molecular masses of the ligand—cross-linked receptors by subtraction of the 19-kD IL-4 or the 15-kD IL-13 (Fig 7a). The majority of [125I]IL-4 was cross-linked at the 130-kD protein, whereas a double band was detected at 65 to 75 kDa. Different traces of radioactivity were also detected at higher molecular weights. The [125I]IL-13 predominantly cross-linked to a protein migrating at 65 to 75 kDa. A trace amount of radioactivity bound at the 130-kD level, presumably to the IL-4Ra (Fig 7a). Addition of 1 μmol/L of each unlabeled cytokine completely displaced its iodinated counterpart, indicating the specificity of the IL-4 and IL-13 binding. One micromolar concentration of unlabeled IL-13 completely displaced the binding of [125I]IL-4 at 65 to 75 kD and most of it at 130 kD. Similarly, 1 μmol/L cold IL-4 abolished [125I]IL-13 binding (Fig 7a).

Immunoprecipitation of the [125I]IL-4—cross-linked membrane proteins with the IL-4Ra MoAb S697 and subsequent SDS-PAGE analysis yielded the predicted binding pattern, a predominant band at 130 kD and minor bands at 65 to 75 kD (Fig 7b). No activity was found at the 200-kD protein that presumably consisted of chemically cross-linked aggregates of the two subunits. In cross-linking experiments with [125I]IL-13, the MoAb S697 was unable to precipitate any visible activity (data not shown). As with control IgG, the MoAb TUGh4 against γc failed to precipitate any labeled IL-4 (Fig 7b) and IL-13 (data not shown). The finding that IL-13 did not bind to the transfected B16 cells solely expressing the 130-kD IL-4Ra chain supports the hypothesis of a specific IL-13—binding subunit (Fig 7c). The accessory bands at 120, 55 kD in these experiments, are reported to be precursor forms of the transfected IL-4Ra.30 Together our experiments support the hypothesis of a shared heterodimeric receptor complex where the apparent cross-linking of [125I]IL-13 to the 130-kD band appears to be the consequence of a close complex formation of the 130-kD IL-4Ra and a cognate 65- to 75-kD IL-13-binding subunit.

Signal transduction of the IL-4 and IL-13-induced expression of VCAM-1 on endothelial cells. To explore the signaling pathway downstream of the transmembrane receptor, HUVECs were treated with various kinase inhibitors for 2 hours at 37°C and with 1 nmol/L IL-4 or IL-13 for 16 hours and were then examined for cell surface expression of VCAM-1. Genistein at 4 μmol/L and herbimycin A at 2 μmol/L partially blocked IL-4 and IL-13 effects, whereas total inhibition resulted at a concentration of 15 μmol/L and 10 μmol/L, respectively (Fig 8). At such high concentrations genistein has been shown to inhibit tyrosine kinases and protein kinase C.34 In the endothelial cells a selective action of genistein was considered to be likely, because TNF-induced VCAM-1 expression was still intact. The IL-4 and IL-13 signals were not inhibited by high concentrations of staurosporine (0.1 μmol/L). The lack of inhibition by staurosporine indicates that protein kinase C is not directly involved in the IL-4 and IL-13—mediated signaling (data not shown). Several highly specific and potent tyrosine kinase inhibitors, tyrphostin A47, B46 (1 μmol/L), and wortmannin (10 μmol/L) had no effect. Furthermore, neither the tyrosine phosphatase inhibitor orthovanadate nor okadaic acid, a serine/threonine phosphatase inhibitor, blocked endothelial VCAM-1 induction.

DISCUSSION

The vascular barrier functions in coordinating the leukocyte traffic from the bloodstream to the extravascular tissue. IL-4 has been shown to induce VCAM-1 on endothelial cells, evoking the adherence of VLA-4—expressing leukocytes, particularly eosinophils but not neutrophils.4,10 Such selective localization mediated transendothelial migration of eosinophils in vitro and led to tissue eosinophilia in mice.1,11 By flow cytometry, a variable yet significant population of endothelial cells could be induced to express VCAM-1 in response to IL-4 or IL-13, although all expressed IL-
Fig 6. RT-PCR analysis of the IL-4R subunits in different cell lines, HUVECs, and mouse B16 transfectants overexpressing human IL-4Rα. Twenty micrograms of total RNA were reverse-transcribed and amplified as described in the Materials and Methods. Equal amounts of RT-PCR reaction products were run on a 2% (α-chain and β₂-microglobulin) or 1% (γ-chain) agarose gel containing 0.2 μg/mL ethidium bromide.
Fig 7. Characterization of the endothelial IL-4 and IL-13 receptor by radioligand affinity cross-linking. (A) Confluent HUVECs were detached and labeled with 0.5 nM [125]IL-4 (lanes 1-3) or 3 nM [125]IL-13 (lanes 4-6) before cross-linking was performed using 2.5 mM disuccinimidyl suberate. [125]IL-4 was displaced with a 100-fold excess of unlabeled IL-4 (lane 1) and unlabeled IL-13 (lane 3). Conversely, [125]IL-13 binding was competed with a 100-fold excess of cold IL-4 (lane 4) and IL-13 (lane 6). (B) Lysed HUVECs from the cross-linking experiment in (A) were immunoprecipitated with non-binding IgG (lane 1), MoAb TUG4 (lane 2), or MoAb S697 (lane 3). Arrows indicate migrating levels of IL-4Ra (upper left), the IL-13-binding protein (lower left), and Yc (right). (C) Identical cross-linking experiments with [125]IL-13 (lane 1) and [125]IL-4 (lane 2) were performed with mouse B16 cells transfected with human IL-4Ra cDNA. The lysates were separated on a gradient (3% to 10%) SDS-PAGE, run under reducing conditions, and exposed to x-ray films for 3 weeks at −70°C. Net molecular masses of the receptors (R), on the left side of the picture, were calculated by subtracting 19 kD for bound IL-4 or 15 kD for bound IL-13.

Fig 8. Inhibition of the IL-4 and IL-13-induced VCAM-1 expression by the kinase inhibitors herbimycin A and genistein. HUVEC monolayers were preincubated for 2 hours either with medium containing DMSO (1:1,000 [vol/vol]), herbimycin A, or genistein at indicated concentrations before either the indicated cytokines or an identical volume of buffer was added to the HUVECs for 16 hours. Numbers are the percentage of cells within the indicated margins (M1) representing the immunopositive population. The results are from one representative experiment using the same batch of endothelial cells.

In immunofluorescence studies, we observed clonal expression of VCAM-1 in response to IL-4 (unpublished observation) leading to focal adherence and transendothelial migration of eosinophils. Regardless whether HUVECs were exposed to IL-4, IL-13, or both, the VCAM-1—expressing population was of the same size and of comparable intensity. Moreover, IL-4 and IL-13 possess a similar capacity to evoke transendothelial migration of eosinophils. However, on a molar basis HUVECs were about 15 times more sensitive to IL-4.

Binding studies with the HUVECs and ECV304, a transformed cell line derived from HUVECs, showed a single class of binding sites with similar high affinity for IL-4 (≃30 pmol/L) and IL-13 (≃60 pmol/L). In the experiments with HUVECs, IL-4 and IL-13 bound to approximately 200 and 700 sites per cell, respectively. COS-3 and A431 cells are reported to have an IL-4 affinity of ≃20 pmol/L, whereas T lymphocytes express two receptor populations, one with a kd value of ≃100 pmol/L and a second with low binding affinity. These variable affinities may account for differences in the receptor configuration. The affinity of IL-13 on COS-3 and A431 cells was ≃300 pmol/L. These investigators reported that IL-13, after iodination of the single tyrosine (Tyr4), almost completely lost its bioactivity. Therefore, they used a modified form of IL-13 (Phe431L-13–GYYG) that was suitable to be iodinated by the chloramin T method. In our study IL-13 was labeled by the iodogen method that completely maintained the activity of native IL-13. The same procedure has also been successfully used by others. This directly labeled IL-13 bound to the HUVECs and ECV304 with an affinity comparable with that of IL-4.
In radioligand cross-linking experiments with different cell lines, the iodinated IL-4 consistently labeled proteins at 220, 145, and 70 kD; whereas IL-13 was cross-linked only to two proteins at 220 and 70 kD. By this technique, endothelial cells showed a banding pattern in which IL-4 was predominantly cross-linked to IL-4Ra, with a net molecular mass of ≈130 kD, and to a minor yet unknown protein migrating at 65 to 75 kD. IL-13 bound predominantly to the 65- to 75-kD protein, although a minor band was observed at 130 kD, presumably the IL-4Ra. There was no radioactive band identified at 220 kD. Supporting the data obtained with different cell lines, high concentrations of cold IL-4 completely displaced labeled IL-13 from its binding to the HUVECs. Despite the fact that the majority of radiolabeled IL-4 was removed by cold IL-13, a trace amount of labeled IL-4 was still detectable at 130 kD. On different cell lines, the capacity of cold IL-13 to remove labeled IL-4 was highly variable. Such cross-competition of IL-4 and IL-13 has been suggested to result from ligation of shared subunits of a multimeric receptor complex that varies in its configuration. Supportive of this model is our finding that, in IL-4 cross-linking experiments, the anti-IL-4Ra MoAb S697 was able to coprecipitate both the 130-kD and the 65- to 75-kD band. S697 did not, however, precipitate visible bands from IL-13–cross-linked endothelial cells, and IL-4Ra–transfected B16 cells did not bind labeled IL-13. Together, our data suggest that there is a weak association between the IL-4Ra and the IL-13–binding subunit and no intimate contact between IL-13 and the IL-4Ra.

The IL-4R and the IL-7R were initially described as single-chain receptors. Very recently the γ-chain of the IL-2R has been described as a common subunit, slightly increasing the affinity of these receptors for their respective ligands. Although the γ-chain associates with the 130-kD IL-4R, it does not directly bind IL-4. Moreover, these studies have inferred a function for the γc in signal transduction. It has been proposed that the IL-13R uses the γc as a receptor subunit. Indeed, most of the cell lines involved in IL-4 and IL-13 receptor studies contained the γc, including those that responded to IL-13. In contrast, we show that human endothelial cells do not express γc on their surface and that no message was found at the mRNA level with sensitive RT-PCR.


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Interleukin-4 (IL-4) and IL-13 bind to a shared heterodimeric complex on endothelial cells mediating vascular cell adhesion molecule-1 induction in the absence of the common gamma chain

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