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Conservation of Structure and Function Between Human and Murine IL-16

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IL-16 is a proinflammatory cytokine that signals via CD4, inducing chemotactic and immunomodulatory responses of CD4+ lymphocytes, monocytes, and eosinophils. Comparative analysis of murine and human IL-16 homologs could reveal conserved structures that would help to identify key functional regions of these cytokines. To that end, we cloned the murine IL-16 cDNA and found a high degree of amino acid similarity comparing the predicted murine and human IL-16 precursor proteins (pro-IL-16). The highest similarity (82.1%) was found in the C-terminal region, which is cleaved from pro-IL-16 to yield biologically active IL-16. Chemotaxis experiments with IL-16 of murine and human origin, using murine splenocytes or human T lymphocytes as targets, showed cross-species stimulation of motility. Synthetic oligopeptides and anti-peptide Ab were produced, based on the sequences of three predicted hydrophilic domains of IL-16 potentially presented in exposed positions. None of these peptides had intrinsic IL-16 bioactivity, but one (corresponding to a hydrophilic C-terminal domain of IL-16) partially displaced binding of OKT4 mAb to human lymphocytes. This peptide, and its cognate Ab, also inhibited IL-16 chemoattractant activity for human and murine cells. These studies demonstrate a high degree of structural and functional similarity between human and murine IL-16 and suggest that amino acids in the C terminus are critical for its chemoattractant function. The data suggest cross-species conservation of IL-16 receptor structures as well. Inhibitory peptides may be useful in disease states where the proinflammatory functions of IL-16 are detrimental to the host. The Journal of Immunology, 1998, 160: 5945–5954.

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J.K. and J.N. made equal contributions to the work reported in this manuscript. Their names are listed in alphabetical order.

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Abbreviation used in this paper: RACE, rapid amplification of cDNA ends.
bacteriophage Agt10. The other cDNA library was prepared from BALB/c mouse lung using both oligo(dT) and random priming, and the cloning vector was also bacteriophage Agt10. Both libraries were screened by plaque hybridization using 32P-labeled probes prepared by nick translation of the 2150-bp human IL-16 cDNA (Promega, Madison, WI). One clone was isolated from 105 plaques of the spleen library but was found to be incompletely expressed. A partially overlapping cDNA clone was isolated from the lung library, providing additional 5' sequence information. Human and murine IL-16 cDNA sequences were analyzed using Lasergene software packages (DNASTAR, Madison, WI) for predicted amino acid sequence similarity by the method of Lipman and Pearson (10), hydrophilicity profiles by the method of Kyte and Doolittle (11), and surface probability by the method of Emuin (12). Potential coding regions were assessed by Fickett's test code (13) in the Wisconsin Package Version 9.0 (Genetics Computer Group, Madison, WI).

Recombinant human and murine IL-16

Recombinant human IL-16 was produced in Escherichia coli as a polyhistidine fusion protein containing 130 C-terminal residues encoded by the previously reported human cDNA (6), using the pET16b vector (Novagen, Madison, WI). It was purified by metal chelation chromatography, and the histidine tag was cleaved with factor Xa. Recombinant β-galactosidase was produced and purified in an identical fashion for use as a negative control. Recombinant murine IL-16 was produced in E. coli as a polyhistidine fusion protein containing 118 C-terminal residues encoded by a murine cDNA, cloned into the pET30 vector (Novagen). It was purified by metal chelation chromatography, and the histidine tag was cleaved with enterokinase.

Natural murine IL-16

Natural murine IL-16 was isolated by incubating 2 × 106 mouse splenocytes with Con A (2 µg/ml) for 48 h (37°C, 5% CO2) before harvesting supernatant and cell lysate. Supernatant was concentrated 10-fold using a Centricron-3 filter (Amicon, Beverly, MA). Splenocytes were lysed by incubation on ice for 3 min in lysis buffer (PBS containing 1% Nonidet P-40, 0.02% NaN3, 1 mM PMSF, and 10 µg apoprotinin). A polyclonal rabbit anti-human IL-16 Ab, raised against a 130 amino acid human rIL-16 produced in E. coli, was covalently bound to staphylococcal protein A-conjugated Sepharose beads (Pharmacia Biotech, Piscataway, NJ) for use in affinity chromatography. Stimulated murine splenocyte supernatant was applied to the column for 1 h, then washed. Ab-bound protein was specifically eluted into one tenth volume 1 M Tris buffer (pH 8.8) using glycine buffer (pH 4.0), then quantitated by BCA assay (Pierce, Rockford, IL).

Peptides and Abs

Synthetic oligopeptides corresponding to three hydrophilic domains identified within the human IL-16 sequence were synthesized. The sequence of peptide 1 (SLEGKGSGLHGD) corresponds to amino acids 546 to 557 of pro-IL-16. The sequence of peptide 2 (ASEQSETVQPGD) corresponds to amino acids 570 to 581. The sequence of peptide 3 (RRKSLQSKETTAAGDS) corresponds to amino acids 615 to 630. After conjugation to keyhole limpet hemocyanin, each peptide was used for rabbit immunization. Anti-peptide IgG Ab were obtained from immunized rabbit sera by column chromatography using staphylococcal protein A-conjugated Sepharose beads (Pharmacia), then specifically purified with CNBr-linked peptide Sepharose columns. Polyclonal rabbit anti-human IL-16 was raised against the 130 amino acid human rIL-16 and purified in a similar fashion on protein A and immobilized rIL-16 columns. Polyclonal rabbit anti-human IL-16 was produced as a monochonal anti-human IL-16 (14.1) were verified by Western blotting with rIL-16 and shown to be neutralizing, as demonstrated by inhibition of chemoattractant activity, IL-2R expression, and HIV-1 repression in IL-16-treated cells (6, 14).

Northern blot analysis

A commercial multiple tissue murine Northern blot (Mouse MTN; Clontech) consisting of 2 µg poly(A)+ RNA per lane from specific tissues transferred from a formaldehyde/1% agarose gel onto a positively charged nylon membrane was probed with a 32P-labeled fragment of the murine IL-16 cDNA. The membrane was treated for 1 h with a prehybridization solution (QiuxHybe; Strategene, La Jolla, CA) containing 10 mg/ml salmon sperm DNA, and subsequently hybridized with the cDNA probe for 1 h overnight at 68°C. After hybridization, the blot was washed twice at low stringency conditions of 2× SSC (300 mM NaCl, 30 mM sodium citrate, 0.5% sodium pyrophosphate, and 1% sodium laurel sarcosine) at room temperature, followed by a wash at high stringency of 0.1× SSC at 60°C. Hybridization was visualized by autoradiography.

Western blot analysis

Affinity-purified native murine IL-16 (40 µg) and recombinant IL-16 were applied to SDS/15% polyacrylamide gels, then transferred to nitrocellulose by electroblotting (3500 Vh, 4°C). The nitrocellulose was incubated 2 h in PBS with 1% lactalbumin and 0.05% Tween 20, then probed with 125I-conjugated polyclonal anti-human rIL-16 Ab added at 1 µg/ml for 1 h. After washing in PBS, the dried blots were visualized autoradiography overnight at room temperature.

Immunoprecipitation

Anti-IL-16 Abs were incubated with rIL-16 protein at 4°C for 4 h under gentle rotation. Protein A-Sepharose beads were added, and the mixture incubated for an additional 1 h. The beads were washed three times with PBS, then resuspended in SDS-PAGE sample buffer and boiled. Released material was analyzed on a 15% SDS-polyacrylamide gel. After electrophoresis, proteins were transferred to nitrocellulose membrane for immunoblotting analysis.

Chromotaxis assay

Chromotaxis was performed using a modified Boyden chamber assay as described (1, 2). Cells were suspended at 5 × 105/ml in complete medium. A 12 µm nitrocellulose membrane separated cells in the upper wells from control buffer or experimental supernatants in the lower wells. Chambers were incubated at 37°C for 4 h, then the membranes were removed, stained with hematoxylin, and dehydrated by sequential washes in ethanol, propa- nal, and then xylene. Cell migration was quantitated by light microscopy, counting the number of cells migrating below a depth of 50 µm. Counts were compared with control (unstimulated) migration, which was normalized to 100%. All samples were tested in duplicate, and four high power fields were examined in each duplicate. Results were analyzed using Student’s t-test, and a P value < 0.05 was considered significant.

Flow cytometry

To test the capacity of IL-16-derived peptides to inhibit anti-CD4 mAb binding, 2 × 105 human T lymphocytes were incubated with 10 µg of each peptide for 2 h at room temperature, then incubated with 10 µg of FITC-conjugated OKT4 or OKT4A mAb (Ortho Diagnostics, Raritan, NJ) for 30 min. Cells were then washed twice in PBS and resuspended at 1 × 106 cells/ml, fixed with 10% formalin, and stained in the dark at 4°C before analysis with a Becton Dickinson FACScan 440 (Becton Dickinson, Sunnyvale, CA) as described previously (15). Induction of IL-2R on IL-16-stimulated murine splenocytes was detected by staining with FITC-conjugated anti-mouse IL-2R Ab (PharMingen, San Diego, CA).

Results

Murine IL-16 cDNA cloning and comparison of predicted human and murine IL-16 proteins

Using fragments of human IL-16 cDNA as probes, we isolated murine IL-16 cDNA clones from bacteriophage Agt10 libraries of mouse spleen and lung (GenBank accession no. AF006001). Figure 1 shows the protein sequence predicted from the longest open reading frame of murine IL-16. This 624-amino acid murine putative pro-IL-16 is aligned with a human pro-IL-16 based on sequence data that we derived by a combination of RACE and genomic DNA cloning (GenBank accession no. M90391), as well as corrected DNA sequence analysis of our published human IL-16 cDNA clone (6). Our findings are in substantial agreement with the human pro-IL-16 sequence reported by Baier et al. (7), differing at only four residues (Glu424 to Asp, Arg232 to Gly, Thr233 to Phe, and Ala319 to Glu). The findings are also consistent with the demonstration of ~80-kDa and ~67-kDa bands by Western blot analysis of human PBMC lysate probed with an anti-human IL-16 Ab (7, 16), suggesting the expression of a human pro-IL-16 in vivo.

Human IL-16 mRNA potentially encodes a 631-amino acid precursor protein (Fig. 1). A pro-IL-16 of 605 amino acids would be produced if the downstream and in-frame Met28 were used as the start site of translation. At both of these potential start sites, the Met residues are weak initiator codons according to the scanning model for translation, lacking key nucleotides normally present in
FIGURE 1. Alignment of the predicted human and murine pro-IL-16 amino acid sequences. The sequence of the originally reported 2150-bp human IL-16 cDNA (6) was extended by 5’ RACE, and cDNA clones of murine IL-16 were isolated as described in Materials and Methods. Alignment of the predicted amino acid sequences for the longest open reading frames in both the human (hu) and murine (mu) IL-16 cDNAs are shown. Between each residue of the two sequences a vertical bar represents a perfect match, a colon represents a favorable mismatch (weight table value 0), a period represents a neutral mismatch (weight table value 1), and a blank space represents an unfavorable mismatch (weight table value < 0).

The mature, biologically active, secreted form of IL-16 is derived by cleavage at Asp510 , yielding a 121-residue monomer. We have recently published evidence that cleavage at this site is mediated by caspase-3 in human lymphoid tissue (18). The caspase-3 cleavage site is conserved in murine pro-IL-16, while Met 35 is not. Potentially, either or both of the two upstream regions, vs 75.1% for the remaining N-terminal precursor sequences. Methionine residues at positions 258 and 502 of pro-IL-16 were previously postulated to be start sites for translation of human IL-16 (6, 19, 20). In the murine sequence, Met 502 is replaced by Thr. Met 258 is also not conserved in certain simian IL-16 homologs (20), indicating that it is very unlikely to serve as an initiator for human IL-16. In contrast, human Met 258 is conserved (as Met 254 ) in the predicted murine pro-IL-16 sequence. Whether this Met residue could serve as an alternative translational start site that might account for the reported appearance of a ~60-kDa natural human IL-16 band on immunoblot remains to be determined (7).

Two GLGF sequence motifs present in human IL-16 are conserved in the murine homolog. One of these sequences lies within the precursor region (G522 to F525) and the other lies within the predicted murine pro-IL-16 sequence (G 542 to F 545 ). This motif is contained within a domain pattern designated PDZ (previously called Discs-Large homology repeats) which are generally involved in intracellular protein-protein interactions (21, 22). Alignment of the upstream and downstream IL-16 PDZ domains (Fig. 2) indicates they are more closely related to one another (Lipman-Pearson similarity index 33.3%) than to PDZ domains in other intracellular proteins, suggesting unique interactions. Any function for these PDZ domains, or for the other highly conserved regions within other precursor sequences of IL-16, is currently unknown.

Northern blot analysis of murine tissues, probed with a 32P-labeled fragment of murine IL-16 cDNA, revealed strong signals from spleen and lung (Fig. 3). In a separate Northern blot experiment, an identical strong signal was present in thymus (data not shown). The relatively high constitutive expression of IL-16 mRNA in mouse spleen and thymus is consistent with the primarily lymphoid tissue distribution and constitutive expression of human IL-16 mRNA. The strong IL-16 signal in mRNA from mouse lung contrasts with normal human lung where IL-16 mRNA is barely detectable. However, markedly increased expression of IL-16 mRNA (and protein) in bronchial epithelium of human asthmatics has been identified (23). It is unknown if the cells expressing IL-16 have been identified (23). It is unknown if the cells expressing IL-16 mRNA in mouse lung are of lymphoid (e.g., bronchus-associated lymphoid tissue) or epithelial origin. Murine IL-16 mRNA expression also differs from that seen in human lymphoid tissues in that it appears as two predominant forms of ~2.5 kb and ~3.5 kb, whereas a single predominant band at ~2.7 kb is found in human tissues. The two mRNA species in the mouse may arise from usage of different polyadenylation signals, but this remains to be determined.

Biologic activity of recombinant murine IL-16

Recombinant murine IL-16 corresponding to the 118 C-terminal residues of the precursor protein was expressed in E. coli. Nucleotide sequence of different pro-IL-16 cassettes was expressed in E. coli. Nucleotide sequence of different pro-IL-16 cassettes was expressed in E. coli. Nucleotide sequence of different pro-IL-16 cassettes was expressed in E. coli. Nucleotide sequence of different pro-IL-16 cassettes was expressed in E. coli. Nucleotide sequence of different pro-IL-16 cassettes was expressed in E. coli. Nucleotide sequence of different pro-IL-16 cassettes was expressed in E. coli.
induced IL-2R expression on a fraction of resting murine splenocytes (Fig. 4B), and this activity was blocked when cells were stimulated with murine rIL-16 in the presence of anti-IL16 Ab (data not shown).

Previous experiments analyzing natural and recombinant human IL-16 by Sephadex G-100 molecular sieve chromatography and HPLC indicated that the monomeric IL-16 peptides must form aggregates, presumably noncovalently associated homotetramers, to exhibit bioactivity (2;6). We have postulated that tetrameric IL-16 induces CD4 signaling by cross-linking CD4 receptors (5).

Recombinant murine IL-16 (predicted molecular mass 12,234 Da) was subjected to HPLC, and column fractions were tested for chemotactant activity. Peak activity was eluted in the column fraction corresponding to a molecular size of 60 kDa (Fig. 4C). The HPLC column fractions were tested for the presence of IL-16 by ELISA, and it was detected only in the 60-kDa range column fractions, which had also demonstrated IL-16 bioactivity. Together, these data are consistent with our earlier findings and indicate that, like human IL-16, murine IL-16 monomers undergo spontaneous noncovalent association to form tetramers that are required for biologic activity.

Identification and functional characterization of natural murine IL-16

Previous studies from our laboratory indicated that an intracellular pool of biologically active IL-16 is stored in human CD8+ lymphocytes and that IL-16 represents the majority, if not all, of the chemotactant activity that can be recovered from lysate of unstimulated human lymphocytes (25). Based on these observations, and the high level of sequence conservation between murine and human IL-16, we predicted that testing unstimulated murine leukocytes for the presence of preformed chemotactant factors capable of inducing motility of human target cells would provide a strong bias for the identification of natural murine IL-16. As shown in Figure 5A, crude lysate of unstimulated BALB/c mouse splenocytes induced a motile response by human PBMC (175% mean % control migration ± SEM, p = 0.05). This activity was strongly inhibited by an Ab raised against a 130-amino acid human rIL-16. The specificity of this anti-IL-16 Ab for neutralizing IL-16 chemotactant activity was previously confirmed in experiments where it was found not to inhibit motility induced by MIP-1α, MIP-1β, or RANTES (26). Similar to our previous findings with human lymphocytes, there appears to be a preformed pool of biologically active murine IL-16 in resting splenocytes.

A column for affinity chromatography was constructed by linking anti-human rIL-16 Ab to staphylococcus protein A-conjugated Sepharose beads, and this was used for partial purification of natural murine IL-16. Affinity-purified supernatant of Con-A-stimulated mouse splenocytes was subjected to Western blot analysis using 125I-conjugated anti-human rIL-16 Ab as probe, and a band of ~17 kDa was identified (Fig. 5B). This is similar to the SDS-PAGE mobility of murine rIL-16 (not shown) as well as recombinant and natural human IL-16 (6). The migration of IL-16 on SDS-PAGE appears to be somewhat aberrant, since rIL-16 produced in E. coli, and therefore unmodified, has a predicted molecular mass of ~13 kDa yet migrates as ~17 kDa. These functional and immunoblot results strongly suggest that the protein eluted from the affinity column was the natural secreted murine IL-16 homolog.
Cross-species bioactivity of IL-16

To further test the hypothesis that the murine and human IL-16 homologs would demonstrate cross-species functionality, human rIL-16 and affinity-purified natural murine IL-16 were assayed for the induction of motility using either murine splenocytes or human T lymphocytes as responding cells. The results presented in Figure 6, A and B, show that both the murine and the human cytokines are chemoattractant for either murine or human target cells in a dose-dependent manner. Peak motility was induced at IL-16 concentrations in the $10^{-10}$ M range, and high dose inhibition (a characteristic of chemoattractant cytokines) was observed with both preparations. While murine IL-16 appeared to be marginally more potent for murine cells, and human IL-16 appeared to be more potent for human cells, the differences in motility in these experiments were not significant.

Identification of structural requirements for IL-16 biologic activity

Kyte-Doolittle hydrophilicity (11) and Emini surface probability plots (12) were generated, based on the predicted amino acid sequence of secreted human IL-16 (Fig. 7A). This analysis indicated the presence of three hydrophilic domains (labeled 1, 2, and 3, respectively from the N terminus to the C terminus). The surface probability prediction was highest in the region corresponding to the hydrophilic domain 3 located at the C-terminal end of the protein, although it was also positive in the central hydrophilic domain 2. A very similar pattern was observed in plots based on murine IL-16. We postulated that one or more of these domains would be exposed on the surface of naturally folded IL-16, and thus likely to be involved in CD4 binding. Synthetic oligopeptides corresponding to each of the three hydrophilic domains were produced, and Ab against these three synthetic oligopeptides were raised in rabbits. Of these three anti-peptide Ab, anti-peptide 2 and anti-peptide 3 were capable of detecting rIL-16 on Western blots, and anti-peptide 3 immunoprecipitated rIL-16 (Fig. 7B).

Previous work from our laboratory indicated that the anti-CD4 mAb OKT4 binds to an epitope on CD4 near the domain involved in IL-16 recognition. We therefore tested the effect of the three IL-16-derived oligopeptides on the binding of FITC-conjugated OKT4 and OKT4A mAb to human T lymphocytes. Figure 8 shows...
the result of flow cytometry experiments where peptide 3, but not peptides 1 or 2, partially displaced the binding of OKT4. The binding of OKT4 was not affected by any of the three oligopeptides. This result suggests that peptide 3 might physically associate with CD4.

We next tested the effect of the synthetic IL-16 peptide fragments on rIL-16-stimulated lymphocyte motility. In these experiments, peptide 3 partially inhibited the chemotactic activity of both human rIL-16 and affinity-purified murine natural IL-16 for human target cells (Fig. 9, A and B). The human rIL-16-stimulated motility of 192% ± 18% (mean % control migration ± SEM) was reduced to 144% ± 14%, and the murine natural IL-16-stimulated motility of 185% ± 15% was inhibited in the presence of peptide 3 to 130% ± 12% (p < 0.05). In contrast, peptides 1 and 2 (derived from the N-terminal and central hydrophilic peaks of human IL-16) demonstrated little or no blocking activity in these experiments. Since peptide 3 appears to compete with the anti-CD4 mAb OKT4, inhibition of IL-16 biologic activity by this peptide is postulated to be due to competition with IL-16 for CD4 binding. This is an important distinction since inhibition of IL-16 bioactivity would also be predicted to occur if multimer formation was disrupted, thus preventing receptor cross-linking. The question whether peptide 3 could neutralize IL-16 by disrupting autoaggregation was further assessed by HPLC. Autoaggregation of rIL-16 monomers to the biologically active tetrameric form was previously confirmed by the demonstration of chemotactic activity only in HPLC column fractions corresponding to ~60 kDa (Fig. 4C). Preincubation of rIL-16 with peptide 3 did not alter this peak of activity (data not shown), suggesting that neutralization of biologic activity was not a result of disaggregation of tetrameric IL-16.

The effect of rabbit anti-peptide Ab on human IL-16-stimulated motility of human T lymphocytes was also tested (Fig. 9C). In these experiments, the rIL-16-stimulated motility of 190% ± 22% was reduced to 111% ± 18% in the presence of anti-peptide 3.
Abs to peptides 1 and 2 demonstrated no blocking effect. Finally, peptides 2 and 3, and their corresponding anti-peptide Ab, were tested on murine splenocytes stimulated with murine rIL-16. Peptide 3 and anti-peptide 3 inhibited murine rIL-16-stimulated splenocyte migration, similar to their blocking effects against human IL-16 (Fig. 9D). None of these peptides or Ab, when used alone, affected cell motility. Taken together, these data suggest that the C-terminal hydrophilic domain of IL-16 might be involved in CD4 binding and is critical for the expression of IL-16-stimulated lymphocyte motility.

Discussion

We isolated cDNA clones of the murine IL-16 homolog and compared them with a full-length human IL-16 cDNA sequence derived by 5′ RACE and genomic cloning. Similar to the 631-amino acid putative human pro-IL-16, murine IL-16 appears to be produced as a precursor protein of up to 624 amino acids. The biologically active and secreted mature form of IL-16 is derived by caspase-3 cleavage of the C-terminal region from pro-IL-16 (18). Comparing the predicted amino acid sequences of murine and human IL-16, there is a high degree of homology throughout the putative pro-IL-16 molecule, but the highest conservation is found in the C-terminal region that is postulated to be cleaved and secreted. Recombinant murine IL-16, based on the Asp506 cleavage site and expressed in E. coli, demonstrated chemoattractant activity and IL-2R induction similar to human IL-16, and these activities were neutralized by anti-human IL-16 Ab. Furthermore, murine rIL-16 was shown to undergo autoaggregation, forming homotetramers required for biologic activity, a unique property of IL-16.

Analysis of crude lysate of unstimulated murine splenocytes revealed the presence of natural murine IL-16 in a preformed intracellular storage pool. Preformed bioactive IL-16 has previously been identified in unstimulated human CD8+ T cells (25). Anti-human rIL-16 Ab was used for immunoaffinity purification of the secreted form of natural murine IL-16 from supernatant of Con A-stimulated splenocytes. Affinity-purified murine IL-16 demonstrated chemoattractant activity similar to human IL-16; it was blocked by neutralizing anti-human rIL-16 Ab, and its mobility on SDS-PAGE was similar to human IL-16. Functional cross-reactivity between murine and human IL-16 was observed in experiments with natural murine IL-16 and human rIL-16, both of which stimulated motility in both human and murine mononuclear cells. The antigenic and functional cross-reactivity of these proteins is consistent with the...
high degree of structural similarity revealed by molecular cloning of the murine IL-16 cDNA.

Previous experiments suggested that the anti-CD4 mAb OKT4 binds to an epitope that is proximate to, but probably not identical with, the domain of CD4 that interacts with IL-16. Fab fragments of OKT4 have been shown to inhibit IL-16 in a variety of functional assays including chemotaxis, IL-2R induction, and signal transduction (intracellular calcium flux and inositol triglyceride generation; (6, 15)). Theodore et al. reported that coincubation of T lymphocytes with IL-16 resulted in partial displacement of OKT4, but not OKT4A mAb assessed by flow cytometry (14). Similarly, Maciaszek et al. found that OKT4 mAb partially inhibited IL-16-mediated repression of HIV-1 promoter activity in CD4+ lymphoid cells (27). In the present studies (based on the hydrophilicity and surface probability plots of human and murine IL-16) we identified two regions within the secreted cytokine likely to be exposed on the surface and thus possibly involved in receptor binding. A third N-terminal hydrophilic domain did not have a corresponding high surface probability prediction, making it a less likely candidate for receptor binding. Synthetic oligopeptides representing the amino acid sequences of these three regions in the predicted human IL-16 protein sequence were tested for their ability to inhibit OKT4 and OKT4A mAb binding to lymphocytes. Only the peptide that corresponded to the C-terminal hydrophilic domain of IL-16 (peptide 3) was found to partially displace OKT4, while none of the peptides displaced OKT4A. Consistent with the studies of IL-16 and OKT4 binding cited above, the inhibitory activity of peptide 3 was incomplete. Presumably, this sixteen-residue peptide would provide less steric interference with OKT4 binding than the 130-amino acid rIL-16. The ability of the three IL-16 peptides (and anti-peptide Ab) to neutralize IL-16 bioactivity was tested by chemotaxis assay. Consistent with the FACS data, peptide 3 demonstrated significant IL-16 blocking ability in experiments with human rIL-16 and natural as well as recombinant murine IL-16 stimulating human or murine target cells. Anti-peptide 3 Ab was also a potent inhibitor. Since autoaggregation is required for IL-16 to exert biologic effects, a peptide or Ab that disrupted this association could also function as an IL-16 inhibitor. However, chemotaxis assays of HPLC column fractions of rIL-16 preincubated with peptide 3 provided no evidence of interference with IL-16 tetramer formation. This finding, together with the observation that peptide 3 partially

FIGURE 9. Inhibition of IL-16 biologic activity by a synthetic oligopeptide and anti-peptide Ab. A. Human T lymphocytes were stimulated with 1.4 ng/ml human rIL-16 alone, or in the presence of 10 μg each of three IL-16-based oligopeptides described in Materials and Methods and indicated in Fig. 6. Results are expressed as the mean % control migration ± SEM for three experiments. The asterisk indicates a significant difference in migration (p < .05) of cells treated with peptide 3 plus human rIL-16, compared with cells stimulated by human rIL-16 alone. B. Human T lymphocytes were stimulated with 1.4 ng/ml affinity-purified natural murine IL-16 alone, or in the presence of 10 μg each of the three IL-16 oligopeptides. The asterisk indicates a significant difference in migration (p < .05) comparing cells treated with peptide 3 plus natural murine IL-16, compared with cells stimulated by murine IL-16 alone. C. Inhibition of IL-16 by anti-peptide 3 Ab. Human T lymphocytes were stimulated with 1.4 ng/ml human rIL-16 alone, or in the presence of 10 μg each of three Ab raised against the IL-16-based synthetic oligopeptides. Results are expressed as the mean % control migration ± SEM for three experiments. The asterisk indicates a significant difference in migration (p < .05) of cells treated with anti-peptide 3 Ab plus human rIL-16, compared with cells stimulated by IL-16 alone. D. Inhibition of murine IL-16-induced murine splenocyte migration by IL-16 peptides and anti-peptide Ab. Murine splenocytes were stimulated with 1.4 ng/ml murine rIL-16 alone, or in the presence of 10 μg each of peptide 2, peptide 3, anti-peptide 2 Ab, or anti-peptide 3 Ab. Results of one experiment are expressed as mean % control ± SD where four high power fields were counted in duplicates of each condition.
displaced OKT4 binding to CD4, suggests that the C-terminal region of IL-16 may be involved in CD4 binding. In these experiments, peptide 3 is postulated to function as a competitive inhibitor for receptor binding, while anti-peptide 3 presumably blocks the receptor-binding domain on IL-16. The cross-species chemotactic activity of murine and human IL-16, and the ability of peptide 3, which is based on the human IL-16 sequence, to block stimulated migration of murine target cells, also suggests interspecies conservation of the CD4 domain, which interacts with IL-16.

Sequence comparison between human and murine IL-16 provides additional insight to the structural and functional features of these cytokines. Although the mechanism of processing and secretion of murine IL-16 has not been characterized, conservation of sequences surrounding Asp506 (corresponding to Asp510 in human pro-IL-16) is consistent with our observation that cleavage of human pro-IL-16 is mediated caspase-3 (18). While a high degree of similarity in the secreted murine and human IL-16 sequences is readily understood in terms of their known cytokine properties, the functional significance of regions of high similarity within the residual N-terminal precursor domains is unclear. An intracellular function for pro-IL-16 was hypothesized, based on the presence of PDZ domains (19), although there is presently no functional data to support that concept. While the IL-16 precursor sequences could play a role in the regulation of processing and secretion, most of this region appears not to be absolutely required for IL-16 release. We originally isolated a truncated IL-16 cDNA encoding 374 residues of pro-IL-16 by expression cloning, yet the COS cells transfected with this construct secreted only the C-terminal portion (6). Similarly, Zhou et al. reported that transfection of Jurkat cells with a vector expressing only 130 C-terminal residues of IL-16 resulted in secretion of biologically active cytokine into the culture medium (28).

The data presented here indicate a high degree of functional conservation between murine and human IL-16, consistent with their sequence homology. Both human and murine mononuclear cells respond to either human or murine IL-16 in the Boyden chamber chemotaxis assay. Furthermore, a requirement for autoaggregation to occur for murine IL-16 to exert biologic activities indicates that it functions in a similar fashion to human IL-16. A wealth of data indicates that multimeric human IL-16 activates cells by signaling through CD4, and it is hypothesized that receptor cross-linking occurs upon binding by these multimers (5). Our results suggest that murine IL-16 acts via an identical mechanism. It is unknown whether other cell surface molecules in addition to CD4 are required for IL-16 binding. However, transfection of L3T4+ murine hybridoma cells with human CD4 alone is sufficient to enable IL-16-stimulated signal transduction and activation responses in these cells (6). The ability of human IL-16 to induce chemotaxis in murine splenocytes, and for murine IL-16 to act in a similar fashion on human T lymphocytes, also suggests that involvement of a second receptor molecule is unlikely.

IL-16 has been found to exert diverse activities via CD4 signaling in lymphocytes, monocytes, and eosinophils. Originally identified by its chemotactic properties (1), IL-16 was subsequently found to induce signal transduction via CD4, to up-regulate IL-2R and HLA-DR expression, and to increase RNA content consistent with a G0 to G1a shift in resting CD4+ T cells (6, 15, 24, 35). In addition to these activation and competence growth factor properties, IL-16 has been found to exert certain inhibitory effects, including inhibition of one way MLR, inhibition of Ag and anti-CD3 Ab-induced T lymphocyte proliferation, and repression of HIV-1 promotor activity (5, 14, 27). The circumstances in which each of these activities is physiologically significant in vivo remains to be determined. The capacity of IL-16 to signal through CD4 and to prime resting CD4+ T lymphocytes to respond to IL-2 raises the possibility of a role in T lymphocyte development, and the chemotactic properties of IL-16 appear to be important in the pathophysiology of certain inflammatory diseases characterized by tissue accumulation of CD4+ lymphocytes, monocytes, and eosinophils. Conditions where IL-16 has been identified by ELISA and/or bioassay of body fluids, or by immunohistochemical and in situ hybridization techniques, include bronchial asthma (23, 26), inflammatory bowel disease (36), Graves’ disease (37), multiple sclerosis (38), and bullous pemphigoid (39). The ability to study IL-16 in murine model systems should facilitate understanding its roles in normal and pathologic immune function. We show here that a murine IL-16 homolog is expressed and that its structure and functions are very closely related to the known properties of human IL-16. It is therefore likely that murine model systems will be suitable for studies using hybridization and immunohistochemical techniques, as well as treatment with murine rIL-16 and IL-16 inhibitors (like peptide 3) to provide information relevant to IL-16 functions in humans.

References


