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Cutting Edge: CD4 Is Not Required for the Functional Activity of IL-16

Nathalie L. Mathy, Norbert Bannert, Stephen G. Norley, and Reinhard Kurth

IL-16 functions as a chemoattractant factor, inhibitor of HIV replication, and inducer of proinflammatory cytokine production. Previous studies have suggested that CD4 is the receptor for IL-16, because only CD4+ cells respond to IL-16 and both the anti-CD4 Ab OKT4 and soluble CD4 can block IL-16 function. However, these are only indirect evidence of a requirement for CD4, and to date a direct interaction between IL-16 and CD4 has not been shown. In this paper, we report that cells from CD4 knockout mice are as responsive to IL-16 as their CD4 wild-type equivalents in both assays testing for IL-16 function (chemotaxis and production of proinflammatory cytokines). In addition, the inhibitory effect of soluble CD4 on IL-16 function observed using CD4 wild type murine cells was not observed using CD4 knockout cells. These data demonstrate that CD4 is not required for IL-16 function and suggest that another molecule acts as the major receptor. The Journal of Immunology, 2000, 164: 4429–4432.

Materials and Methods

Production of recombinant mouse IL-16 (rmIL-16)

Murine IL-16 cDNA was ligated into the Escherichia coli expression vector pET-16b, which is under the control of the isopropyl-thiogalactopyranoside-inducable lacUV5 promoter (Novagen, Bad Soden, Germany). The IL-16-(His)6 fusion protein was purified using the B-Per 6× His fusion protein purification kit (Pierce, St. Augustin, Germany) and then was cleaved with Factor Xa (Promega, Mannheim, Germany) to remove the polyhistidine tag. Endotoxin was removed using Detoxigel endotoxin-removing gel (Pierce). rmIL-16 preparations contained <0.1 U endotoxin/10 µg protein and were used at a final concentration of 100 or 500 ng/ml for assays.

Animals

All animal experiments were performed under paragraph 8 of the German Animal Protection Law (Tierschutzgesetz) in compliance with European Commission Directive 86/609, which makes it an offence to carry out any scientific procedure on an animal except under license. For the studies of IL-16 function in normal murine cells, CD4 wild-type (CD4+/+) C57BL/6 mice were used. CD4−/− mice were obtained from The Jackson Laboratory (Bar Harbor, ME). These mice, originally described by Rahemtulla et al. (7), are also of C57BL/6 background and do not express any CD4 on the cell surface. Blood was obtained from anesthetized mice (Metofane; Janssen, Neuss, Germany) by cardiac puncture after which the animals were sacrificed by cervical dislocation.

In vitro cell culture

Fresh murine PBMCs were isolated by Ficoll/Hypaque gradient centrifugation. Total PBMCs were cultured at 1 × 10^6 cells/ml in RPMI 1640 supplemented with 10% heat-inactivated FCS, 4 mM L-glutamine, and 100 µg/ml penicillin/streptomycin (crPMI) supplemented with or without rmIL-16 or with LPS (5 µg/ml; Sigma, Deisenhofen, Germany) for 24 or 48 h. To test for the production of other cytokines, rmIL-16 was used at a concentration of 100 or 500 ng/ml diluted in cRPMI, whereas for chemotaxis experiments, a concentration of 100 ng/ml was used. Negative controls received media alone. Monocytes were isolated or depleted from the total PBMC population by positive or negative selection, respectively, using CD11b magnetic cell separation system beads (Miltenyi Biotec, Germany) by cardiac puncture after which the animals were sacrificed by cervical dislocation.

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3 Abbreviations used in this paper: CD4+/+, CD4 knockout; rmIL-16, recombinant mouse IL-16; CD4+/−, CD4 wild type.
samples with a neutralizing anti-IL-16 mAb (0.2 μg/ml per 15 ng/ml IL-16; PharMingen, Germany) or with soluble CD4 (5 μg/ml per 500 ng/ml IL-16; Intracel, Germany). Each experiment was performed a minimum of six times and yielded similar results.

Cytokine sandwich ELISA

Mouse IL-1β, IL-6, and TNF-α protein levels were detected by sandwich ELISA according to the manufacturer’s instructions (R&D Systems, Wiesbaden, Germany). The minimum detectable concentrations were 3 pg/ml for mIL-1β, 3 pg/ml for mIL-6, and 5 pg/ml for mTNF-α. Student’s t test was used for statistical analysis to identify differences between samples using the mean values. Significance was established at the p < 0.05 level of confidence.

Chemotaxis

The chemotactic activity of rmIL-16 was assessed by quantification of cell migration using Transwell chamber inserts (5-μm membrane size; Costar, Germany) according to the manufacturer’s instructions. Briefly, CRPMI medium, containing rmIL-16 (100 ng/ml), was added to the cluster plate. Total PBMCs were added to the Transwell at a concentration of 1 × 10⁶ cells/ml, which was then inserted into the plate and incubated for 2 h at 37°C in a 5% CO₂ atmosphere. The Transwell was removed and the number of cells in the lower well was counted using a Coulter counter (Coulter, Germany). Specificity for IL-16 was tested by incubating test samples with a neutralizing anti-IL-16 mAb (PharMingen). Each set of experiments was performed a minimum of four times with triplicate samples and yielded similar results. Student’s t test was used for statistical analysis to identify differences between samples using the mean values. Significance was established at the p < 0.05 level of confidence.

Results

IL-16 induces the secretion of proinflammatory cytokines by mouse monocytes

Total PBMCs from CD4⁺/⁺ mice were cultured with rmIL-16 (100 or 500 ng/ml), and supernatants were tested for the presence of IL-1β, IL-6, and TNF-α by ELISA after 24 and 48 h. Although there was very little to no IL-1β production using either rmIL-16 concentration after 24 or 48 h of culture (Fig. 1A), IL-6 and TNF-α were produced after 24 h (Fig. 1, B and C). After 48 h of culture with rmIL-16, levels of secreted IL-6 and TNF-α were similar to those seen at 24 h (data not shown). The amounts of each cytokine produced in response to rmIL-16 were much lower than when the cells were stimulated with LPS (Fig. 1).

Total PBMCs depleted of monocytes using magnetic beads did not produce IL-6 and TNF-α in response to rmIL-16 (data not shown). In contrast, monocytes purified from PBMCs readily produced IL-6 and TNF-α when cultured with 500 ng/ml rmIL-16 for 24 h (Fig. 2, B and C). However, very little to no IL-1β could be detected (Fig. 2A). Similar levels of the three cytokines were detected after 48-h stimulation with rmIL-16 (data not shown). The
use of an anti-IL-16 Ab inhibited production of IL-1β, IL-6, and TNF-α, as did the addition of soluble CD4 (data not shown).

In experiments performed on PBMCs isolated from CD4+/+ BALB/c mice, similar results to those observed for the C57BL/6 strain were obtained (data not shown).

Cells devoid of CD4 expression respond to IL-16

To directly assess the requirement of CD4 for IL-16 function, PBMCs from CD4−/− mice were isolated and stimulated with rmIL-16 for 24 h, after which supernatants were tested for IL-1b by ELISA. As can be seen in Fig. 1, IL-1β (in low quantities), IL-6, and TNF-α were readily detected (Fig. 1, D–F). Indeed, the concentrations were generally higher than those obtained using cells from CD4+/+ mice (Fig. 1, A–C). Interestingly, the addition of soluble CD4 did not inhibit production of the three cytokines by the CD4−/− cells (Fig. 3).

To address the need for CD4 in IL-16-induced cell migration, a chemotaxis assay was performed using PBMCs from the CD4+/+ and the CD4−/− mice. Migration of cells was seen in samples from both the CD4+/+ and CD4−/− mice (Fig. 4), and the use of an anti-IL-16 Ab inhibited this migration (data not shown).

Discussion

IL-16 plays a number of roles in the immune system. Its presence in high concentrations in alveolar lavages of asthmatic patients and its ability to induce the production of proinflammatory cytokines suggests a role in inflammation (8, 9). IL-16 has also been shown to inhibit HIV replication and acts as a chemoattractant factor (4, 6). Similar to results using human monocytes (5), we demonstrate in this study that murine monocytes also produce the proinflammatory cytokines IL-6 and TNF-α upon IL-16 stimulation. However, unlike their human counterparts, murine monocytes produce very little to no IL-1β. Human monocytes were also found to produce IL-15 upon IL-16 stimulation (5), although this has yet to be tested for the murine cells.

Although a number of in vitro experiments have suggested the need for CD4 in the activity of IL-16 (3, 6, 10), to date there has been no direct evidence of an interaction between the two. We decided to use CD4−/− mice to determine whether IL-16 does indeed require the presence of CD4 to function. These CD4−/− mice express no CD4 on the surface of their cells and are also deficient in Th cells (7). Although CD4 is strongly expressed on the surface of monocytes (11), these knockout mice have levels of CD14+ peripheral monocytes similar to those of their wild-type equivalents (N. L. M., unpublished observations). The fact that PBMCs lacking the CD4 receptor react as well as, if not better than, CD4+/+ cells in terms of both cytokine production and chemotaxis is strong evidence that CD4 is not a requirement for the IL-16 function. This also shows that CD4+ T cells are not responsible for the IL-16 activity seen.

This direct evidence for a lack of CD4 involvement is in contrast to the indirect evidence suggesting that IL-16 binds the CD4 molecule. We and other groups have shown that IL-16 activity can be blocked using an anti-CD4 Ab (OKT4) or even soluble CD4 (6) when using PBMCs containing CD4+ cells (i.e., wild type). Blocking by OKT4, which binds the D4 domain of CD4 (12), suggests that when access to CD4 is denied, IL-16 cannot bind to the cell and be active. However, as CD4 is not required for IL-16 activity (as shown here), it is possible that the real receptor for IL-16 lies in close proximity to CD4 and that binding of the OKT4 Ab prevents interaction with IL-16 by steric hindrance. The addition of soluble CD4 to the CD4-deficient system does not result in the inhibition of IL-16 activity seen using CD4+/+ cells. This strongly suggests that the soluble CD4 does not bind directly to the IL-16 to prevent its function. Previous studies on CD4 have shown that CD4 can self-associate (12, 13), and this may be happening on the surface of CD4+/+ cells when soluble CD4 is added to the system. Both CD4 self-association and the OKT4 (anti-CD4) Ab require the D4 domain of CD4 (6, 12), and it is therefore possible that binding to this domain prevents interaction between IL-16 and its actual receptor by steric hindrance. Alternatively, inhibition of IL-16 activity could be occurring via an indirect intracellular signal after complexing of cellular CD4 by OKT4 or soluble CD4.

The results presented here clearly show that CD4 is not required for the functional activity of IL-16 on mouse cells. In addition, the results also indicate that the inhibition of IL-16 activity by soluble CD4 or OKT4 Ab is indirect and that CD4 does not bind to IL-16. Although this study was unavoidably performed using the murine system, there is no reason to believe that the situation in humans would be any different.

Acknowledgments

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References