The IL-2 Receptor γ_c Chain Does Not Function as a Subunit Shared by the IL-4 and IL-13 Receptors[1]

Implication for the Structure of the IL-4 Receptor

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The IL-2 receptor (IL-2R) γ_c subunit is also a component of the receptors for IL-4, IL-7, IL-9, and IL-15. The IL-4R and IL-13R appear to share a common subunit, and γ_c was proposed to be this shared subunit. In this study, we have assessed the relative contribution of γ_c to the mouse IL-4R and IL-13R. The MC/9 mast cell line constitutively expresses γ_c and proliferates to IL-4 and IL-13, but only the response to IL-4 was blocked by anti-γ_c mAbs. After transfection of the IL-4- and IL-13-responsive γ_c-negative B9 plasmacytoma with full length (mγ) or cytoplasmic-tailless γ_c cDNA (mγt), only the proliferative response to IL-4 was affected by the surface expression of these γ_c molecules. The inability of mγ or mγt expression to affect IL-13-induced proliferation by B9 indicates that γ_c does not obviously contribute to the IL-13R and does not function as the shared subunit of the IL-4R and IL-13R. This study suggests that there are two distinct IL-4R, one of which is independent of γ_c. ***The Journal of Immunology,*** **1995, 155: 9–12.**

The IL-2 receptor (IL-2R) is comprised of three distinct subunits, α, β, and γ (1). The α-chain functions to enhance IL-2 binding, whereas the β and γ subunits participate in binding as well as signaling upon interaction with IL-2. Recently, the IL-2R γ subunit has been identified to be a component of the receptors for IL-4, IL-7, IL-9, and IL-15 (2–7). Because of its shared nature, the γ subunit has been designated as γ_c (2).

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IL-13, a T cell-derived cytokine, shares many biological functions with IL-4. Like IL-4, IL-13 promotes growth of preactivated human B cells (8), suppresses the production of proinflammatory cytokines by activated monocytes (9), and induces $p57^{lsk}$ tyrosine kinase expression in monocytes (10). Although the IL-13R has not been characterized, the relationship between the IL-4R and IL-13R has been studied by binding and functional experiments with a mutant IL-4 protein, $IL\text{-}4_{Y124D}$, which binds to IL-4Rα but does not signal. $IL\text{-}4_{Y124D}$ was shown to inhibit both IL-4- and IL-13-induced IgG4 and IgE synthesis and B cell proliferation (11). IL-13 competitively inhibited IL-4 binding to its receptor, but it did not bind to the IL-4Rα expressed on COS7 cells (12). These observations led to the conclusion that the receptors for IL-4 and IL-13 share a common component. This shared component has been widely speculated to be γ_c (2, 3, 13–15).

In a previous study, we found that the B9 plasmacytoma, which responds to IL-4 and IL-13, does not express γ_c at both the mRNA and protein levels, indicating that the biological response to these cytokines was not absolutely dependent upon γ_c (16). This study was performed to examine the relative contributions of γ_c to the IL-4R and IL-13R.

Materials and Methods

Cell lines

Cells were cultured in RPMI 1640 containing 5% fetal calf serum, glutamine (30 μg/ml), penicillin (100 U/ml), streptomycin (100 μg/ml), and β-mercaptoethanol (5×10^{-5} M) (complete medium). The B9 mouse plasmacytoma and MC/9 mouse mast cell (kindly provided by Dr. G. Zurawski, DNAX Research Institute, Palo Alto, CA) were maintained in 10% IL-6 or IL-3 conditioned medium, respectively. To test for clonal variation, B9 cells were subcloned. All of the resulting 11 B9 subclones responded to IL-4 and IL-13. A B9 subclone with relatively high proliferation to IL-4 and IL-13 was chosen for subsequent transfections. cDNAs encoding full-length or truncated mouse γ_c were directionally subcloned into the *Xho*I/*Not*I sites in the eukaryotic expression vector BCMGSneo (16). The truncated γ_c cDNA encodes the extracytoplasmic and transmembrane domains and two amino acids of the intracytoplasmic tail of the mature peptide. The B9 subclone was transfected with these vectors by electroporation as previously described to yield B9mγ and B9mγt transfectants (17). These cells were maintained in complete medium containing G418 (1 mg/ml; Life Technologies, Inc., Gaithersburg, MD).

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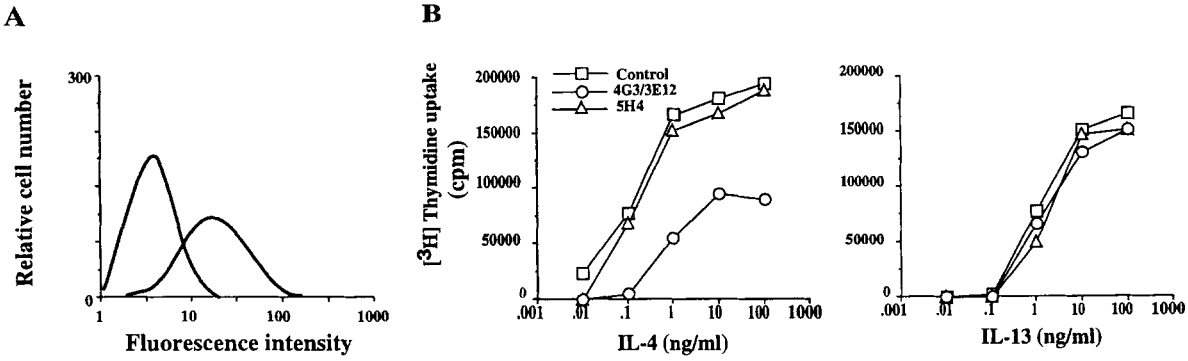

FIGURE 1. Expression and function of γ_c in the MC/9 mast cell line. *A*, FACS analysis of γ_c expression in MC/9. MC/9 cells were incubated with excess of biotinylated 4G3 and followed by staining with PE-streptavidin. The control represents cells stained with only PE-streptavidin and is shown as the peak to the left. *B*, Effect of anti-γ_c mAbs on IL-4- and IL-13-induced proliferation by MC/9 cells. MC/9 cells were incubated with mouse recombinant IL-4 or IL-13 with the indicated mAbs (1:200 dilution of ascites) for 40 h.

Biological reagents

The 4G3 (rat IgG2a) and 3E12 (rat IgG2b) mAbs to the mouse IL-2Rγ_c and the 5H4 (rat IgG2a) mAb to the mouse IL-2Rβ chain were produced in our laboratory and described elsewhere (16, 18). Mouse recombinant IL-4 was obtained from PeproTech, Rocky Hill, NJ. Mouse recombinant IL-13 was kindly provided by DNAX, Palo Alto, CA.

Flow cytometry

Cells were subjected to indirect FACS analysis by incubation of cells with an excess of biotinylated 4G3 followed by staining with phycoerythrin (PE)-conjugated streptavidin (Fisher Biotech) as previously described (19).

Cytokine bioassays

Cells (5×10^3/well) were cultured in complete medium containing the appropriate cytokine as indicated for 40 h at 37°C in a humidified atmosphere containing 7% CO_2. [^{3}H]thymidine (1 μCi/well; 25 Ci/mmol; Amersham, Arlington Heights, IL) was added during the last 10 h of culture. The labeled DNA was harvested on glass fiber filters and counted in a beta scintillation counter. Data is presented as the mean of triplicate determinations that varied by less than 10% of the mean.

Northern blot analysis

Northern blot analysis was performed essentially as previously described (20), using a full length [^{32}P]cDNA probe to mouse γ_c.

Results

Effect of anti-γ_c mAbs on IL-4- and IL-13-induced proliferation of MC/9 mast cells

The MC/9 mast cells are maintained by culture with IL-3, but these cells also proliferate to IL-4 and IL-13. This cell line was shown to express γ_c by FACS analysis with the anti-γ_c mAb 4G3 (Fig. 1*A*). To assess the potential role of γ_c in IL-4 and IL-13 induced proliferation by MC/9, we examined the effect of anti-γ_c mAbs 4G3 and 3E12, reactive with distinct epitope of γ_c, on the proliferation induced by these two cytokines. Previous work has shown that 4G3 preferentially inhibits IL-7 bioactivity, whereas 3E12 preferentially inhibits IL-4-induced responses; cultures containing a mixture of these two mAbs were somewhat more inhibitory for both IL-4- and IL-7-induced proliferation (16). As expected, IL-4-induced proliferation by MC/9 was readily inhibited by a mixture of 4G3 and 3E12 at all concentrations of IL-4 (Fig 1*B*). By contrast, the proliferation of MC/9 to IL-13 was not affected by these mAbs (Fig 1*C*). As a control, the noninhibitory 5H4 mAb to the IL-2Rβ chain did not affect these responses. These data indicate that γ_c participates in IL-4-induced signaling by MC/9 and raise the possibility that IL-13-induced proliferation is independent of γ_c.

Role of γ_c in IL-4 and IL-13 induced proliferation of B9 plasmocytoma

We have previously demonstrated that the B9 plasmacytoma does not express γ_c, yet proliferates to IL-4 and IL-13 (16). To assess the relative contribution of γ_c to the IL-4R and IL-13R, a subclone of B9 was transfected with either the full length mouse γ_c (mγ) or the mutant γ_c (mγt) cDNA that lacks most of the cytoplasmic tail. FACS analysis of independent transfected cell lines demonstrated that cells transfected with full length (B9mγ) or truncated (B9mγt) γ_c cDNA expressed cell surface γ_c; whereas this protein was not detected on the surface of untransfected cells (Fig. 2*A*). The cell surface level of γ_c on B9mγ was similar to that seen for MC/9, while much higher levels of the truncated γ_c were observed on B9mγt. The expression of full-length and truncated γ_c in these cell lines was confirmed by Northern blot analysis that revealed the expected 1.2 or 0.85 kb bands, respectively (Fig. 2*B*).

A role for γ_c in the IL-4R was clearly shown in these B9 transfectants. The half-maximal proliferation of B9mγ to IL-4 occurred at concentration of approximately 0.10 ng/ml, which was a dose-response identical to that seen for MC/9 (Fig. 1*B*). Importantly, B9mγ-1 and B9mγ-2 were 10-fold more sensitive to IL-4 than parental B9. Furthermore, after transfection of mγt cDNA into B9, severe inhibition of proliferation to IL-4 was noted (Fig. 3*A*). Even at 100 ng/ml, the responses to IL-4 by distinct B9mγt transfectants were inhibited by 80 to 95% when compared to untransfected cells. By contrast, transfection of either mγ or mγt into B9 had no

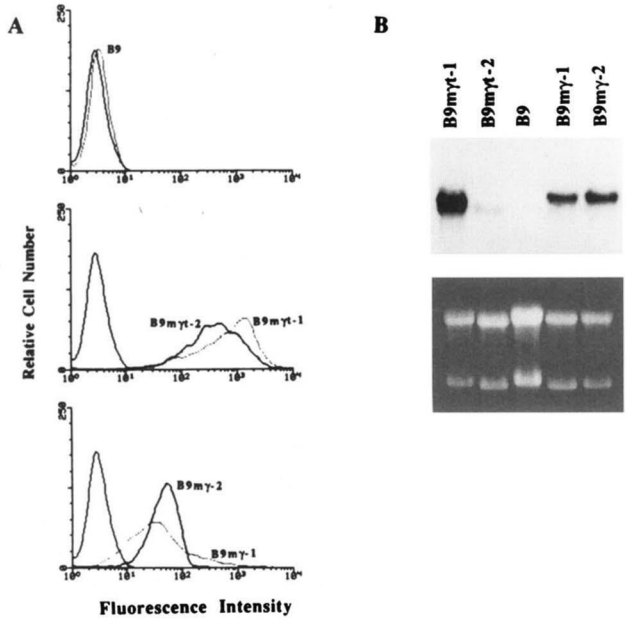

FIGURE 2. Expression of γ_c in B9, B9mγt, and B9mγ. *A*, FACS analysis of γ_c expression in B9 transfectants. Cells as indicated were incubated with excess of biotinylated 4G3 and followed by staining with PE-streptavidin. The control stained cells were incubated with only PE-streptavidin and are always represented by the peak to the left in each histogram. Two independent transfectants for each B9mγ and B9mγt were shown. *B*, mRNA expression of B9 transfectant. Cytoplasmic mRNA (5 μg/*lane*) was subjected to Northern blot analysis from the indicated cell lines and probed with [^{32}P]cDNA to mouse γ_c. The lower panel shows the ethidium bromide staining of the 28s and 18s RNA of the Northern blot.

obvious effect on IL-13-induced proliferation (Fig. 3*B*). The half-maximal response to IL-13 by B9, B9mγt, and B9mγ occurred at the concentration of approximately 0.4 to 0.5 ng/ml. Although the maximum response to IL-13 by B9mγt-1 and B9mγt-2 was somewhat lower than that of B9 and B9mγ, this is probably due to a slower growth rate of B9mγt (Fig. 3*C*).

To further assess the contribution of mγ and mγt on B9, the effect of anti-γ_c mAbs on IL-4- and IL-13-induced proliferation by B9, B9mγ, and B9mγt was examined. As expected, the anti-γ_c mAbs inhibited IL-4-induced proliferation by B9mγ to a level that was similar to that of untransfected B9 cells. These antibodies had no effect on IL-4-induced proliferation of B9mγt, indicating that the mAbs could not reverse the dominant negative blockade of IL-4 responsiveness in these cells (data not shown). Furthermore, the anti-γ_c mAbs did not affect IL-13-induced responses by B9, B9mγ, and B9mγt (data not shown). Taken together, these results showed that γ_c actively participates in IL-4-, but not IL-13-, induced signaling pathway.

Discussion

Recent work has demonstrated that the IL-4R is comprised of two subunits, the 140 kDa IL-4Rα chain and the 75 kDa γ_c subunit (2, 3, 21). A role of γ_c in the IL-4R was established by binding and transfection experiments (2, 22). IL-4 shares not only many structural and functional properties with IL-13 (for review, see Ref. 23), but the receptors for these two cytokines also appear related, as indirect evidence suggests that these receptors share a common subunit (11, 12). There is considerable speculation that this shared component between IL-4R and IL-13R might be the IL-2Rγ_c (2, 3, 13–15). This report provides evidence that the γ_c of the IL-2R does not function as a subunit shared by IL-4R and IL-13R. An implication from these data is that there may be two distinct IL-4Rs.

We have three lines of evidence that supports the notion that γ_c is not the shared component between IL-4R and IL-13R. First, the B9 plasmacytoma, which lacks the γ_c expression, responds to both IL-4 and IL-13. Second, IL-13-induced proliferation of MC/9, a mast cell line that constitutively expresses γ_c, was not affected by mAbs to γ_c, whereas IL-4-induced proliferation was readily inhibited by the mAbs. Third, expression of mγ or mγt cDNA in B9 cells selectively affected IL-4-, but not IL-13-, induced proliferation. A recent study has shown that B cells from X-linked SCID patients do not respond to IL-2 or IL-15, but respond well to IL-4 and IL-13 (14). This result is in further support that the γ_c does not function as the shared component between IL-4R and IL-13R.

One structural model for the IL-4R is that it may exist as a three-subunit complex like IL-2R, namely the IL-4Rα, γ_c, and the subunit shared by IL-4R and IL-13R. Our data suggest that this possibility is highly unlikely, as expression of truncated γ_c on the cell surface of B9 effectively inhibited IL-4-induced proliferation. In a three-subunit model, we expected that expression of mγt might have enhanced the response to IL-4 by increasing ligand binding since the untransfected γ_c-negative B9 cells are fully competent to proliferate to IL-4. The observed strong inhibition by mγt suggests that mγt effectively competes with the subunit shared by IL-4R and IL-13R for association to IL-4Rα, and therefore renders the IL-4R nonfunctional. Due to the high level of mγt expression, we cannot discriminate whether γ_c preferentially associates with IL-4Rα in the presence of the IL-4/IL-13 shared subunit. Transfection experiments by others have also shown that mutant human IL-2Rγ_c had a dominant negative effect on human IL-4R function on a mouse pro-B cell line (22), but in this case the competition was between the mutant human IL-2Rγ_c and the endogenous mouse γ_c.

An important implication, therefore, emerging from this study is that there are two types of IL-4R. One type of IL-4R contains the IL-4Rα and the IL-2Rγ_c subunit (Type I IL-4R) while the other type of the IL-4R may be comprised of IL-4Rα and the shared subunit between IL-4R and IL-13R (Type II IL-4R). Alternatively, Type II IL-4R might consist of the IL-4Rα chain and the IL-13R rather than each receptor sharing a novel subunit.

Type I IL-4R may be exclusively expressed on T cells since IL-13 has not been implicated in the regulation of T cell

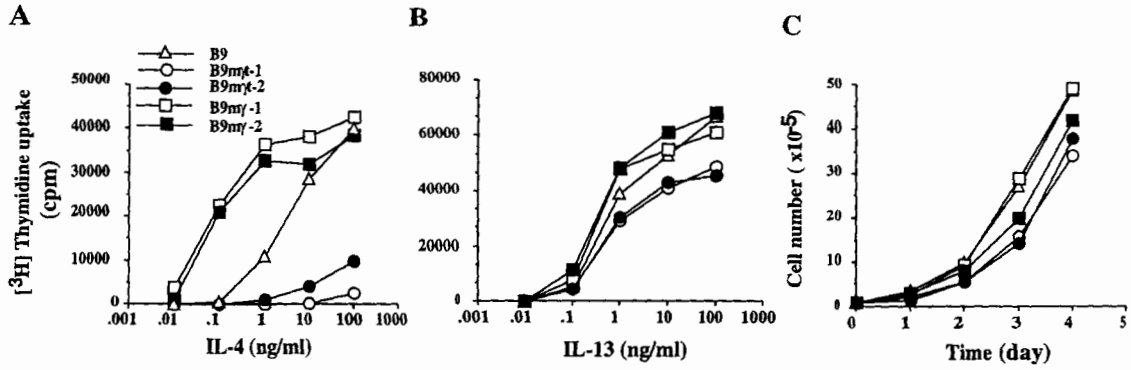

FIGURE 3. Effect of γ_c on cytokine-dependent proliferation by the B9 plasmacytoma. *A*, Proliferation to IL-4. *B*, Proliferation to IL-13. The indicated cells were incubated with IL-4 and IL-13 for 40 h and [^{3}H]TdR was added in the last 10 h of the culture. *C*, Growth to IL-6. B9, B9mγ, and B9mγt cells were maintained in 10% IL-6-conditioned medium, and growth was enumerated by trypan blue exclusion.

bioactivity, supporting the notion that T cells lack functional IL-13R (23). Type II IL-4R may be expressed on IL-13 responsive cells such as human and mouse monocyte/macrophage, human B cells, mouse mast cells, and plasmacytomas. In some situations, these two type IL-4Rs may co-exist on the cell surface, for example, on MC/9 cells. Dose-response studies indicated that Type I IL-4R will likely be functionally active at a lower concentration of IL-4 than that for Type II IL-4R. It is likely that Type I and Type II IL-4R serve distinct regulatory functions. This view is supported by the observation that human lymphocyte expresses high and low affinity IL-4Rs (24) and that IL-4 differentially regulates IgM and CD23 expression on human B cells in the presence of low or high IL-4 concentrations (25).

Note added in proof

Obiri et al. (*J. Biol. Chem. 270:8797*, 1995) and Lin et al. (*Immunity 2:331*, 1995) have recently demonstrated that γ_c is not a subunit of the human IL-13R.

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