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Identification of the second subunit of the murine interleukin-5 receptor: interleukin-3 receptor-like protein, AIC2B is a component of the high affinity interleukin-5 receptor

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Murine interleukin-5 (IL-5) binds to its receptor with high and low affinity. It has been shown that the high affinity IL-5 receptor (IL-5-R) is composed of at least two membrane protein subunits and is responsible for IL-5-mediated signal transduction. One subunit of the high affinity IL-5-R is a 60 kDa membrane protein (p60 IL-5-R) whose cDNA was isolated using the anti-IL-5-R monoclonal antibody (mAb), H7. This subunit alone binds IL-5 with low affinity. The second subunit does not bind IL-5 by itself, and is expressed not only on IL-5-dependent cell lines but also on an IL-3-dependent cell line, FDC-P1. Expression of the p60 IL-5-R cDNA in FDC-P1 cells, which do not bind IL-5, reconstituted the high affinity IL-5-R. We have characterized the second subunit of the IL-5-R by using another anti-IL-5-R mAb, R52.120, and the anti-IL-3-R mAb, anti-Aic-2. The anti-Aic-2 mAb down-regulated binding of IL-5 to an IL-5-dependent cell line, Y16. Both R52.120 and anti-Aic-2 mAbs recognized membrane proteins of 130-140 kDa expressed on FDC-P1 and Y16 cells. The R52.120 mAb recognized both murine IL-3-R (AIC2A) and its homologue (AIC2B) expressed on L cells transfected with suitable cDNAs. The high affinity IL-5-R was reconstituted on an L cell transfectant co-expressing AIC2B and p60 IL-5-R, whereas only the low affinity IL-5-R was detected on a transfectant co-expressing AIC2A and p60 IL-5-R. AIC2B did not bind IL-5 by itself, but was cross-linked with IL-5 in the presence of p60 IL-5-R. These results demonstrate that the murine high affinity IL-5-R is composed of at least two distinct molecules, p60 IL-5-R and the homologue of IL-3-R, AIC2B.

Key words; cytokine/cytokine receptor family/hematopoietic growth factor/IL-5 receptor/lymphokine

Introduction

Interleukin-5 (IL-5), secreted mainly by activated T cells, is a potent regulator of immune responses. IL-5 enhances immunoglobulin production by inducing proliferation and differentiation of activated B cells (Takatsu *et al.*, 1980, 1988; Kinashi *et al.*, 1986; Swain *et al.*, 1988; Tominaga *et al.*, 1991). IL-5 also promotes the growth and differentiation of eosinophils (Yokota *et al.*, 1987; Sanderson *et al.*, 1988; Dent *et al.*, 1990; Tominaga *et al.*, 1991) and enhances histamine release from basophils (Lopez *et al.*, 1990; Hirai *et al.*, 1990). IL-5 manifests its multiple functions via a specific cell surface receptor expressed on various target cells. There are two classes of IL-5 receptor (IL-5-R) in the mouse; one binds IL-5 with low affinity (dissociation constant, K_D ~30 nM), and the other binds IL-5 with high affinity (K_D ~150 pM) and is responsible for IL-5-mediated signal transduction (Mita *et al.*, 1988, 1989).

We have prepared two monoclonal antibodies (mAbs), designated H7 and T21, which completely inhibit the binding of IL-5 to IL-5-responsive cells and recognize a 60 kDa cell surface protein on these cells (Yamaguchi *et al.*, 1990; Hitoshi *et al.*, 1990). We recently isolated a cDNA encoding an IL-5-R from a murine IL-5-dependent early B cell line, Y16, by an expression cloning procedure using H7 and T21 mAbs (Takaki *et al.*, 1990). The murine IL-5-R was shown to be a member of the recently identified cytokine receptor family (Bazan, 1989; Gearing *et al.*, 1989; Itoh *et al.*, 1990). COS7 cells transfected with this cDNA express the 60 kDa protein (p60 IL-5-R) that binds IL-5 with low affinity. However, a functional high affinity IL-5-R is reconstituted by expression of the p60 IL-5-R cDNA in a murine IL-3-dependent hematopoietic cell line, FDC-P1 which does not bind IL-5. These results indicate that the p60 IL-5-R is a component of the functional high affinity IL-5-R, and that an additional molecule(s), which is expressed on FDC-P1 but not on COS7 cells, is involved in the formation of the functional high affinity IL-5-R (Takaki *et al.*, 1990).

In a series of cross-linking experiments with IL-5, we detected two membrane proteins with molecular sizes of ~60 kDa and ~130 kDa on IL-5-dependent cells. The ~130 kDa protein seems to be related to the high affinity IL-5-R (Mita *et al.*, 1989; Yamaguchi *et al.*, 1990). Rolink *et al.* have described the R52.120 mAb which inhibits binding of IL-5 to a murine IL-5-dependent cell line, B13 (Rolink *et al.*, 1989). The R52.120 mAb recognizes membrane proteins of 130-140 kDa different from p60 IL-5-R, and affected binding of IL-5 to the high affinity receptor but not to the low affinity receptor (Mita *et al.*, 1991). Thus, 130-140 kDa proteins recognized by the R52.120 mAb are likely to be the second component of the high affinity IL-5-R.

There are several observations that suggest a close relationship between the IL-5-R and IL-3-R systems. First, several IL-5-dependent cell lines, including T88-M, B13 and Y16, also respond to IL-3 (Tominaga *et al.*, 1989; Rolink *et al.*, 1989). Moreover, IL-3 and IL-5 induce phosphorylation of a similar set of proteins in T88-M cells (Murata *et al.*, 1990). Second, an IL-3-dependent cell line, FDC-P1, becomes responsive to IL-5 when it expresses the

p60 IL-5-R cDNA (Takaki *et al.*, 1990). Third, the R52.120 mAb inhibits IL-3-driven proliferation of B13 (Rolink *et al.*, 1989). Fourth, the R52.120 antigen is also expressed on IL-3-dependent IL-5-non-responsive cell lines, including FDC-P1 (Mita *et al.*, 1991).

In this report, we describe the characterization and identification of a second subunit of the high affinity IL-5-R. An anti-IL-5-R mAb (R52.120) and an anti-IL-3-R mAb (anti-Aic-2) (Yonehara *et al.*, 1990) have been used to establish the relationship between the IL-5-R and IL-3-R systems. The R52.120 mAb recognized the Aic-2 antigens, the murine IL-3-R (AIC2A) which binds IL-3 with low affinity (Itoh *et al.*, 1990), and its homologue (AIC2B) which is considered to be the β chain of the murine GM-CSF-R (Gorman *et al.*, 1990; Kitamura *et al.*, 1991a). Co-expression of p60 IL-5-R with AIC2B, but not with AIC2A, reconstituted the high affinity IL-5-R in L cells. Although AIC2B did not bind IL-5 by itself, it was cross-linked with IL-5 in the presence of p60 IL-5-R. These results demonstrate that the second subunit of the murine IL-5-R is the homologue of the IL-3-R, AIC2B.

Results

Characterization of the functional high affinity IL-5-R reconstituted on the FDC-P1 transfectant

When the p60 IL-5-R cDNA was transfected into FDC-P1, the resulting transfectant (FDC-5R) expressed both high and low affinity IL-5-R and became responsive to IL-5 (Takaki *et al.*, 1990). The responsiveness of FDC-5R to IL-5 was not temporary, and FDC-5R acquired IL-5-dependent proliferative properties in an IL-3-independent manner (Figure 1). Essentially identical results were observed with another transfectant expressing p60 IL-5-R. In contrast, neither the parental FDC-P1 cell line nor a G418-resistant p60 IL-5-R-negative clone (FDC-Neo) bound IL-5 at concentrations up to 4 nM (data not shown) or responded to IL-5 (Figure 1). These results suggest that an additional protein(s) expressed on FDC-P1 may interact with p60 IL-5-R to reconstitute the functional high affinity IL-5-R. To detect and characterize the additional protein(s) on FDC-5R, we performed chemical cross-linking experiments using ^{35}S-labeled IL-5 (Figure 2). Two types of cross-linked complexes of ~100 kDa and ~170 kDa were detected on FDC-5R (lane 3), whereas no cross-linked complex was detected on parental FDC-P1 or FDC-Neo (lanes 5 and 6). This cross-linking pattern was identical to that obtained with Y16 (lane 1). These cross-linked complexes were not found when cross-linking was performed in the presence of an excess amount of unlabeled IL-5 (lanes 2 and 4). After subtraction of the molecular size of IL-5 (40–45 kDa), the sizes of the cross-linked proteins were estimated to be ~60 kDa, which corresponds to that of the cloned IL-5-R gene product, and ~130 kDa. When COS7 cells or L cells transfected with p60 IL-5-R cDNA were cross-linked with ^{35}S-labeled IL-5, only a major cross-linked complex of ~100 kDa was detected (Takaki *et al.*, 1990) (see below). These results further support the notion that a ~130 kDa protein is a component of the high affinity IL-5-R. Moreover, these results indicate that this ~130 kDa protein on an IL-3-dependent cell line does not bind IL-5 by itself but is cross-linked with IL-5 only in the presence of p60 IL-5-R.

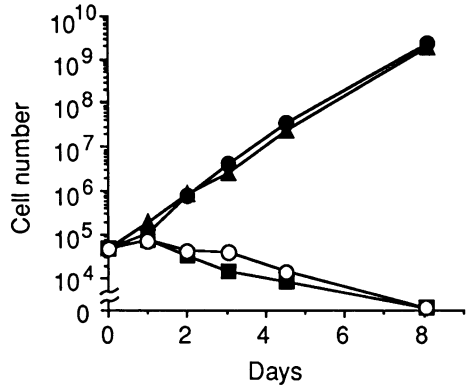

Fig. 1. IL-5-dependent growth of FDC-P1 transfectants expressing the p60 IL-5-R. FDC-P1 and its transfectant clones positive or negative for the p60 IL-5-R were independently cultured in the presence of 10 pM mIL-5. Culture medium was changed every 2 or 3 days, and viable cells were counted. ●, p60 IL-5-R$^+$ transfectant (FDC-5R); ▲, another independent p60 IL-5-R$^+$ transfectant ; ■, G418-resistant, p60 IL-5-R$^-$ transfectant (FDC-Neo); ○, parental FDC-P1.

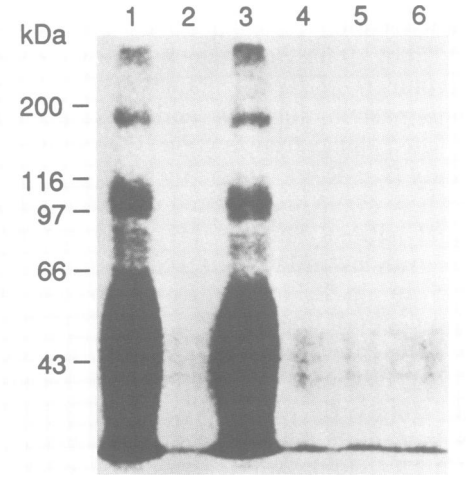

Fig. 2. Chemical cross-linking of ^{35}S-labeled IL-5 to Y16, FDC-P1 and FDC-P1 transfectants. Cells (2×10^7) were incubated with 500 pM ^{35}S-labeled IL-5 at 37°C for 10 min in the absence (lanes 1, 3, 5 and 6) or presence (lanes 2 and 4) of a 100-fold excess of unlabeled IL-5, then cross-linked with DST. Cell lysates were subjected to SDS–PAGE analysis under non-reducing condition and analyzed by BA100. Lanes 1 and 2, Y16; lanes 3 and 4, FDC-5R; lane 5, FDC-P1; lane 6, FDC-Neo. Molecular weight standards are shown on the left.

Common components shared between the IL-5-R and IL-3-R systems

The R52.120 mAb recognizes an antigen distinct from the H7 antigen (p60 IL-5-R) (see Figure 4B) and seems to recognize a second component of the high affinity IL-5-R. Interestingly, we have also found that the R52.120 mAb reacts with murine IL-3-dependent cell lines (FDC-P1 and IC2 (Mita *et al.*, 1991). In addition, the R52.120 mAb had inhibitory effects on proliferation of FDC-P1 cells induced by low concentrations of IL-3 (data not shown). These results suggest that a component of the IL-5-R recognized by the R52.120 mAb is also involved in the IL-3-R system. We considered therefore a possibility that the anti-Aic-2 mAb, which recognizes the low affinity IL-3-R (AIC2A) and its homologue (AIC2B), may have an effect on the IL-5-R

Table I. Effects of anti-Aic-2 mAb on the binding of ^{35}S-labeled IL-5 to Y16 cells

Reagents added in preincubation	^{35}S-labeled IL-5 bound, c.p.m. (%)	
	Preincubated at	
	4°C	37°C
Experiment 1		
None	4012 (100%)	3926 (100%)
Anti-Aic-2	4049 (101%)	3027 (77%)
Unlabeled IL-5	40 (1%)	52 (1%)
Experiment 2		
None	4186 (100%)	3670 (100%)
Anti-Aic-2	4017 (96%)	2211 (60%)
Unlabeled IL-5	55 (1%)	60 (2%)

Y16 cells (2×10^6) were preincubated with medium alone, or 10 μg/ml of anti-Aic-2, or 5 nM unlabeled IL-5 at 4°C or 37°C for 45 min. Cells were then incubated with 50 pM ^{35}S-labeled IL-5 at 37°C for 10 min, and cell-bound radioactivity was measured. The data represent the mean c.p.m. of duplicate determination. In parentheses, the percentage of ^{35}S-labeled IL-5 binding in relation to the control is given.

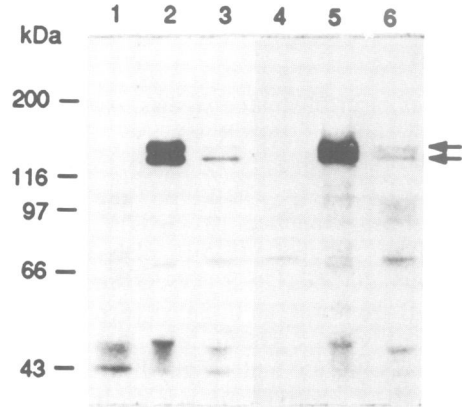

Fig. 3. Biochemical characterization by SDS−PAGE of the antigen recognized by R52.120 (rat IgG_1) and anti-Aic-2 (rat IgM) mAbs. Y16 (lanes 1−3) and FDC-P1 (lanes 4−6) cells were surface-labeled with ^{125}I, and solubilized with PBS containing 1% Triton X-100 and protease inhibitors. Proteins immunoprecipitated with R52.120 mAb (lanes 2 and 5), anti-Aic-2 mAb (lanes 3 and 6), or control rat Ig (lanes 1 and 4) were analyzed by SDS−PAGE under reducing condition. Molecular weight standards are shown on the left.

system. First, we examined the expression of the Aic-2 antigens (AIC2A and AIC2B) on various IL-5 responsive cell lines by flow cytofluorometry, and found that all five IL-5-responsive cell lines tested (Y16, T88-M, BCL_1-B20, MOPC104E and T-88), expressed the Aic-2 antigens (data not shown). Then, we examined whether anti-Aic-2 mAb affects binding of IL-5 to Y16 cells. It has been shown that the anti-Aic-2 mAb (rat IgM mAb) down-regulates Aic-2 antigens following pretreatment of IC2 cells at 37°C, though it does not inhibit binding of IL-3 to IC2 cells at 15°C (Yonehara *et al.*, 1990). Y16 cells were therefore pretreated with anti-Aic-2 at 37°C or 4°C before doing the binding assay. To detect the high affinity binding sites, low concentrations of ^{35}S-labeled IL-5 (50 pM) were employed. As shown in Table I, binding of IL-5 was reduced by 23−40% when pretreatment of Y16 cells with anti-Aic-2

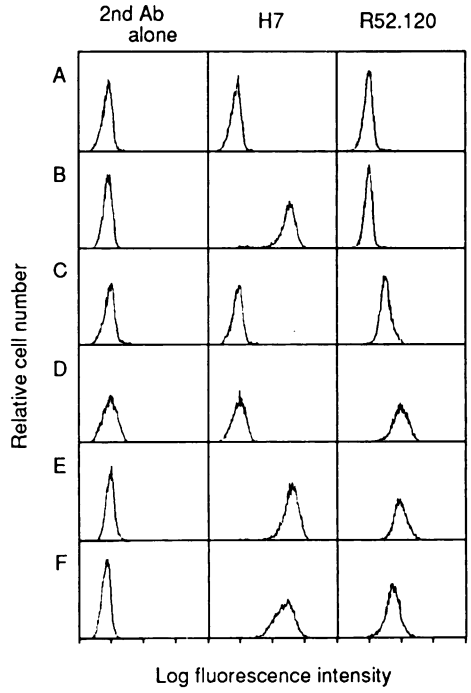

Fig. 4. Cell surface staining pattern of L cells transfected with p60 IL-5-R, AIC2A (mIL-3-R), or AIC2B (the homologue of AIC2A) cDNAs. Cells were separately incubated with H7, R52.120 mAbs, or staining buffer alone. The cells were then incubated with fluorescein isothiocyanate-conjugated $F(ab)'_2$ fragments of goat anti-rat IgG, and subjected to flow cytofluorometry analysis. (**A**), parental Ltk^-; (**B**), L-5R (Ltk^- transfected with p60 IL-5-R cDNA); (**C**), L-2A (Ltk^- transfected with AIC2A cDNA); (**D**), L-2B (Ltk^- transfected with AIC2B cDNA); (**E**), L-5R-2A (L-5R derived clone transfected with AIC2A cDNA); (**F**), L-5R-2B (L-5R derived clone transfected with AIC2B cDNA).

was at 37°C, but not at 4°C, indicating that the anti-Aic-2 mAb down-regulates the number of binding sites for IL-5 at 37°C, but does not compete with IL-5 in binding to Y16 cells.

To characterize the protein recognized by R52.120 mAb further, immunoprecipitation experiments were carried out (Figure 3). Both R52.120 mAb (lanes 2 and 5) and anti-Aic-2 mAb (lanes 3 and 6) immunoprecipitated doublet bands of 130−140 kDa from ^{125}I-labeled Y16 cells (lanes 2 and 3) or FDC-P1 cells (lanes 5 and 6). These results strongly suggest that the R52.120 mAb recognizes the Aic-2 antigens, the low affinity IL-3-R (AIC2A) and/or its homologue (AIC2B).

Reconstitution of the high affinity IL-5-R

The identity of proteins recognized by either R52.120 or anti-Aic-2 mAb was verified using stable L cell transfectants; R52.120 mAb reacted with an L cell transfectant expressing AIC2A (L-2A) (Figure 4C) as well as an L cell transfectant expressing AIC2B (L-2B) (Figure 4D). To explore which of two proteins (or both) is responsible for formation of the high affinity IL-5-R, we cotransfected AIC2A cDNA or AIC2B cDNA with the p60 IL-5-R cDNA into L cells. On an L cell transfectant expressing p60 IL-5-R alone (L-5R), only the low affinity IL-5-R was detected (Figure 5A). L-5R derived clones transfected with AIC2A cDNA (L-5R-2A) or AIC2B cDNA (L-5R-2B) expressed almost equal amounts of the respective cDNA products when assessed by flow cytofluorometry (Figure 4E and F). Neither the K_D value

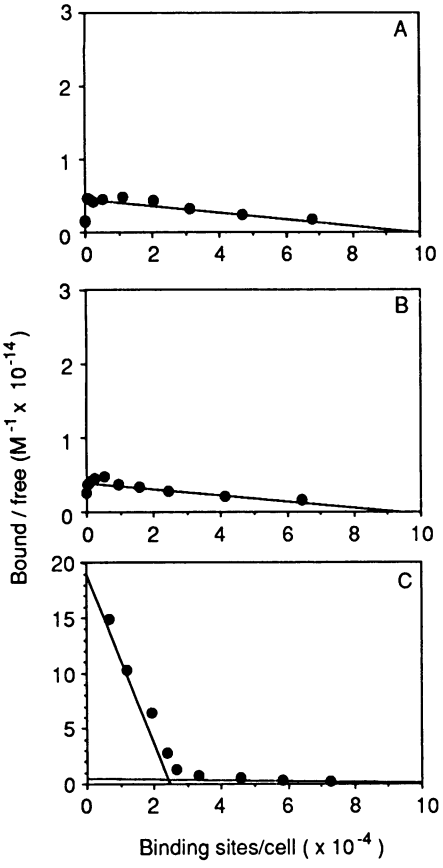

Fig. 5. Scatchard plot analysis of ^{35}S-labeled IL-5 binding to L cell transfectants (**A**), L-5R; (**B**), L-5R-2A; (**C**), L-5R-2B. Cells (1×10^5) were incubated with various amounts of ^{35}S-labeled IL-5 at 37°C for 10 min. Specific binding was determined after subtraction of non-specific binding and the data were expressed as a Scatchard plot. Each cell line was examined twice and the results were reproducible. The number of IL-5 binding sites per cell and the K_D were as follows, (**A**), L-5R, 9.7×10^4 sites per cell, K_D = 2.0 nM; (**B**), L-5R-2A, 9.3×10^4 sites per cell, K_D = 2.3 nM; (**C**), L-5R-2B, 11.0×10^4 sites per cell, K_D = 2.2 nM, 2.4×10^4 sites per cell, K_D = 14 pM.

nor the number of IL-5-Rs on L-5R-2A changed compared with that on L-5R (Figure 5A and B). In contrast, both high and low affinity IL-5-Rs were reconstituted on L-5R-2B (Figure 5C). The K_D values of the reconstituted IL-5-Rs on L-5R-2B were 2.2 nM for the low affinity and 14 pM for the high affinity receptor. These values are similar to those of native IL-5-Rs on various IL-5 responsive cells. L-2A and L-2B did not show specific binding of IL-5 at concentrations up to 4 nM (data not shown).

Cross-linking of ^{35}S-labeled IL-5 to these transfectants confirmed the interaction of p60 IL-5-R with AIC2B (Figure 6). When L-5R or L-5R-2A were cross-linked with ^{35}S-labeled IL-5, only one band of ~100 kDa was detected (lanes 2 and 5). However, when L-5R-2B was cross-linked with ^{35}S-labeled IL-5, the ~170 kDa band appeared in addition to the ~100 kDa band (lane 6). The cross-linking patterns were identical with those in IL-5-responsive cells bearing the high affinity IL-5-R, such as Y16 (Figure 2) and T88-M (Mita *et al.*, 1989). Again, no cross-linked complex was detected on L-2A, L-2B, or parental L cells (Figure 6, lanes 1, 3 and 4). These results show that the homologue of the murine IL-3-R, AIC2B, does not bind IL-5 by itself, but forms the high affinity IL-5-R with the p60 IL-5-R and is cross-linked with IL-5.

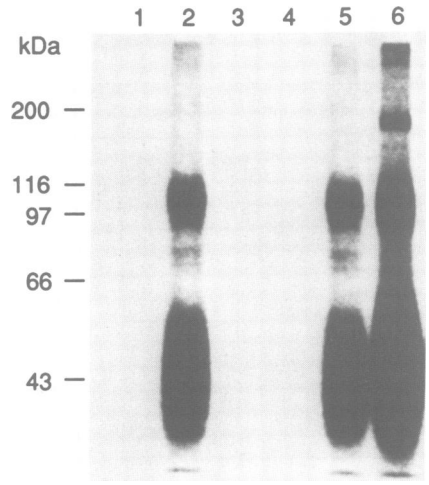

Fig. 6. Chemical cross-linking of ^{35}S-labeled IL-5 to L cell transfectants. Cells (2×10^6) were incubated with 4 nM ^{35}S-labeled IL-5 at 37°C for 10 min, and cross-linked with DST. Cell lysates were subjected to SDS–PAGE analysis under non-reducing condition and analyzed by BA100. Lane 1, Ltk$^-$; lane 2, L-5R; lane 3, L-2A; lane 4, L-2B; lane 5, L-5R-2A; lane 6, L-5R-2B. Molecular weight standards are shown on the left.

Discussion

From a series of cross-linking studies, we have proposed that the high affinity IL-5-R consists of at least two membrane protein subunits, p60 and p130 (Mita *et al.*, 1989; Yamaguchi *et al.*, 1990). The validity of this two subunit model is further supported by cloning and expression experiments of the p60 IL-5-R cDNA. COS7 cells transfected with the p60 IL-5-R cDNA express only the low affinity IL-5-R. However, an IL-3-dependent cell line, FDC-P1, transfected with the p60 IL-5-R cDNA expresses not only low affinity but also high affinity IL-5-Rs and becomes responsive to IL-5 (Takaki *et al.*, 1990). It is likely that an additional component(s) of the IL-5-R, probably p130, is expressed on IL-3-dependent cells and associates with the p60 IL-5-R resulting in the formation of a functional high affinity IL-5-R. The R52.120 mAb (Rolink *et al.*, 1989) seems to recognize this additional component(s) of the IL-5-R (Mita *et al.*, 1991). Since the R52.120 mAb inhibited proliferation of FDC-P1 cells induced by low concentrations of IL-3, the additional component(s) is likely to be involved in the IL-3-R system as well as the IL-5-R system.

The close relationship between the IL-5-R and IL-3-R systems was further supported by using the anti-Aic-2 mAb. The anti-Aic-2 mAb (rat IgM mAb) was originally identified as an anti-IL-3-R antibody that down-regulates binding of IL-3 to IL-3-dependent IC2 cells (Yonehara *et al.*, 1990). However, the cloning of cDNAs encoding the Aic-2 antigen has revealed that the anti-Aic-2 mAb recognizes not only the low affinity IL-3-R, AIC2A (Itoh *et al.*, 1990), but also its homologue AIC2B (Gorman *et al.*, 1990). AIC2B is only 18 amino acid residues longer than AIC2A and has 91% amino acid sequence identity with AIC2A (Gorman *et al.*, 1990). The anti-Aic-2 mAb down-regulated binding of IL-5 to Y16 cells in a manner similar to that observed in the IL-3-R system. Both R52.120 and anti-Aic-2 mAbs immunoprecipitated similar doublet membrane proteins of 130–140 kDa from Y16 or FDC-P1 cells. The identity of

the R52.120 antigen with both AIC2A and AIC2B was clearly demonstrated using L cell stable transfectants expressing these proteins.

The low affinity IL-3-R, AIC2A affected neither the affinity nor the number of low affinity IL-5-Rs. However, the homologue of IL-3-R, AIC2B, interacted with p60 IL-5-R resulting in the formation of the high affinity IL-5-R. The dissociation of IL-5 from L-5R-2B expressing both p60 IL-5-R and AIC2B was much slower than from L-5R expressing only p60 IL-5-R, whereas association kinetics of IL-5 to both L-5R-2B and L-5R were almost similar (data not shown). AIC2B may therefore stabilize the binding of IL-5 to p60 IL-5-R. This is analogous to the formation of the high affinity receptors for IL-2, human GM-CSF (hGM-CSF) and human IL-6 (hIL-6) which are composed of two distinct subunits. One subunit (the α chains of IL-2-R and hGM-CSF-R, and p80 hIL-6-R) binds the specific ligand and behaves as the low affinity receptor by itself (Leonard *et al.*, 1984; Nikaido *et al.*, 1984; Gearing *et al.*, 1989; Yamasaki *et al.*, 1988). The other subunit (the β subunits of IL-2-R and hGM-CSF-R, and gp130 of hIL-6-R) interacts with the low affinity receptor subunit resulting in the formation of the high affinity receptor and is essential for signal transduction (Hatakeyama *et al.*, 1989a,b; Taga *et al.*, 1989; Hibi *et al.*, 1990; Hayashida *et al.*, 1990; Kitamura *et al.*, 1991a). Considering the similarity of the IL-5-R system to these other cytokine receptor systems, we have designated the p60 IL-5-R as the α chain and AIC2B as the β chain of the murine IL-5-R.

Evidence indicating that AIC2B appears to be a subunit of murine GM-CSF-R and essential for signal transduction has been presented recently (Kitamura *et al.*, 1991a). In the mouse, AIC2B appears to be involved in both the IL-5-R and GM-CSF-R systems. However, it is unclear how the signal of IL-5 differs from that of GM-CSF. Two interpretations are likely. First, the signals generated by both cytokines may be equivalent, and different functions of these cytokines may be due to the various developmental stages of cells expressing the receptors. A second interpretation is that different signal transducing molecules, which generate a specific signal for each cytokine, are associated with respective ligand binding subunits (the α chains). These possibilities are not mutually exclusive. It is noteworthy that AIC2B is highly homologous to the low affinity IL-3-R (AIC2A) even in the cytoplasmic domain, and that IL-3, IL-5 and GM-CSF induce phosphorylation of a similar set of proteins (Isfort and Ihle, 1990; Murata *et al.*, 1990). In any case, part of the signal transducing machinery of IL-5 may be shared with GM-CSF, and even with IL-3. Since IL-5 did not induce proliferation of L-5R-2B cells (data not shown), the signal transducing machinery for cell division may not be complete in fibroblasts.

The close relationship among the IL-5-R, GM-CSF-R and IL-3-R systems is also predicted in man. hIL-3 and hGM-CSF compete with each other for binding to some human cells (Park *et al.*, 1989; Lopez *et al.*, 1989). hIL-5 competes with hIL-3 or hGM-CSF for binding to human basophils (Lopez *et al.*, 1990). IL-1 up-regulates the expression of 120 kDa subunits of the hIL-3-R, hIL-5-R and hGM-CSF-R in a similar manner (Kitamura *et al.*, 1991b). Thus, the existence of a common component shared among hIL-5-R, hGM-CSF-R and hIL-3-R is suggested. In comparison with the murine system, we would speculate that the common component is the β subunit of hGM-CSF-R which seems to be the human counterpart of AIC2A and AIC2B (Hayashida *et al.*, 1990). Isolation of the hIL-5-R cDNA may resolve this problem.

In this study, we have demonstrated that the murine high affinity IL-5-R is composed of at least two subunits, p60 IL-5-R (α chain) and the homologue of IL-3-R, AIC2B (β chain). AIC2B is not only highly homologous to the IL-3-R (AIC2A) but is also co-expressed with AIC2A in many cell lines (Gorman *et al.*, 1990). Hence, the following model of receptor systems for differentiation of B cells and eosinophils can be considered. The putative signal transducing subunits (β chains), AIC2A and AIC2B (probably the β chain of hGM-CSF-R in man) are constitutively expressed at an immature, namely, IL-3-responsive stage, and ligand binding subunits (α chains) for IL-5 or GM-CSF are expressed at defined stages during development, creating cells responsive to each differentiation factor. Studying the gene expression pattern and the signal-transducing machinery of receptors for IL-5, GM-CSF and IL-3 will provide an understanding of the mechanisms involved in cell differentiation.

Materials and methods

Cells and reagents

A mouse IL-5-dependent early B cell line, Y16 (Takaki *et al.*, 1990), and a mouse IL-3-dependent cell line, FDC-P1, were maintained in RPMI 1640 medium supplemented with 5% (v/v) fetal calf serum (FCS) and 50 μM 2-mercaptoethanol, in the presence of 10 pM IL-5 or 10 U/ml IL-3, respectively. A mouse fibroblast line Ltk$^-$ was maintained in Dulbecco's modified Eagle's medium supplemented with 10% FCS. H7 mAb (rat IgG_{2a}) (Yamaguchi *et al.*, 1990; Hitoshi *et al.*, 1990) and anti-Aic-2 mAb (rat IgM) (Yonehara *et al.*, 1990) were prepared and purified as described. Purified R52.120 mAb (rat IgG_1) (Rolink *et al.*, 1989) was kindly provided by Dr A.G.Rolink (Basel Institute for Immunology).

IL-5 binding assay

Biosynthetically ^{35}S-labeled murine IL-5 was prepared according to procedures described earlier (Tominaga *et al.*, 1990). For the binding assay, cells were harvested and resuspended at 1×10^5 to 2×10^6 per 100 μl of RPMI 1640 medium containing 25 mM HEPES pH 7.2 and 1 mg/ml bovine serum albumin, and incubated with various concentrations of ^{35}S-labeled IL-5 at 37°C for 10 min. Cell-bound radioactivity was separated from free ligand by centrifugation through 3:2 dibutyl:dioctyl phthalates and counted. Specific binding was defined as the difference between total and nonspecific binding obtained in the presence of 100-fold excess of unlabeled IL-5.

Chemical cross-linking

Chemical cross-linking was performed as previously described (Mita *et al.*, 1989). In brief, cells (2×10^6 to 2×10^7) were incubated with 0.5–4.0 nM of ^{35}S-labeled IL-5 at 37°C for 10 min. The cells were collected and resuspended in 500 μl of Hanks' balanced saline containing 1 mM disuccinimidyl tartarate (DST; Pierce, Rockford, IL). After incubation at 4°C for 30 min, cells were solubilized with lysis buffer (PBS containing 1% Triton X-100, 2 mM EGTA, 2 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 10 μM pepstatin, 10 μM leupeptin, 2 mM *o*-phenanthroline and 200 KIU/ml aprotinin). Cell lysates were then subjected to SDS–PAGE (7.5% polyacrylamide) under non-reducing conditions, and analyzed by BA100 (Fuji Photo Film, Tokyo).

Transfection

The p60 IL-5-R cDNA was inserted downstream of the chicken β-actin promoter of the expression vector pCAGGS (kindly provided by Dr J.Miyazaki, Kumamoto University Medical School), a derivative of pAGS-3 (Miyazaki *et al.*, 1989), resulting in pCAGGS-5R. The cDNAs encoding AIC2A or AIC2B were inserted downstream of the SRα promoter in the expression vector, pME18 (Miyajima *et al.*, unpublished results), a derivative of pCEV4 (Itoh *et al.*, 1990). pCAGGS-5R and pSV2neo (Southern and Berg, 1982) were cotransfected into FDC-P1 and Ltk$^-$ by electroporation using Gene Pulser (Bio-Rad, Richmond, CA) and by a calcium phosphate precipitation procedure, respectively. Transfectants were selected with

400 μg/ml of G418 (Sigma). An Ltk⁻ transfectant expressing p60 IL-5-R (L-5R) was subsequently cotransfected with plasmids containing AIC2A or AIC2B cDNA and pBRtk (Enquist *et al.*, 1979), then selected in HAT medium.

Flow cytofluorometry

L cell transfectants were detached from dishes with PBS containing 0.5 mM EDTA and incubated with PBS containing 0.5 mM EDTA, 5% FCS and mAbs. After washing, cells were stained with fluorescein isothiocyanate-conjugated $F(ab)'_2$ fragments of goat anti-rat IgG (Cappel, West Chester, PA) and analyzed by FACScan (Becton Dickinson, Mountain View, CA).

Immunoprecipitation

Immunoprecipitation was performed as described (Takaki *et al.*, 1990) with some modification. In brief, cells ($\sim 2 \times 10^8$) were radioiodinated using IODO-BEADS (Pierce) and solubilized with the lysis buffer described above. At 4°C, radioiodinated cell lysates were incubated with mAbs or normal rat Ig fraction, and followed by incubation with protein G–Sepharose (Pharmacia) which was pre-incubated with goat anti-rat Ig (Cappel). After extensive washing with saline containing 1% Triton X-100, 0.1% SDS and 50 mM HEPES pH 7.4, immunoprecipitates were subjected to SDS–PAGE (7.5% polyacrylamide) and analyzed by BA100.

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References

Bazan,J.F. (1989) *Biochem. Biophys. Res. Commun.*, **164**, 788–795.

Dent,L.A., Strath,M., Mellor,A.L. and Sanderson,C.J. (1990) *J. Exp. Med.*, **172**, 1425–1431.

Enquist,L.W., Vande Wonde,G.F., Wagner,M., Smiley,J.R. and Summers,W.C. (1979) *Gene*, **7**, 335–342.

Gearing,D.P., King,J.A., Gough,N.M. and Nicola,N.A. (1989) *EMBO J.*, **8**, 3667–3676.

Gorman,D.M., Itoh,N., Kitamura,T., Schreurs,J., Yonehara,S., Yahara,I., Arai,K. and Miyajima,A. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 5459–5463.

Hatakeyama,M., Tsudo,M., Minamoto,S., Kono,T., Doi,T., Miyata,T., Miyasaka,M. and Taniguchi,T. (1989a) *Science*, **244**, 551–556.

Hatakeyama,M., Mori,H., Doi,T. and Taniguchi,T. (1989b) *Cell*, **59**, 837–845.

Hayashida,K., Kitamura,T., Gorman,D.M., Arai,K., Yokota,T. and Miyajima,A. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 9655–9659.

Hibi,M., Murakami,M., Saito,M., Hirano,T., Taga,T. and Kishimoto,T. (1990) *Cell*, **63**, 1149–1157.

Hirai,K., Yamaguchi,M., Misaki,Y., Takaishi,T., Ohta,K., Morita,Y., Ito,K. and Miyamoto,T. (1990) *J. Exp. Med.*, **172**, 1525–1528.

Hitoshi,Y., Yamaguchi,N., Mita,S., Sonoda,E., Takaki,S., Tominaga,A. and Takatsu,K. (1990) *J. Immunol.*, **144**, 4218–4225.

Isfort,R.J. and Ihle,J.N. (1990) *Growth Factors*, **2**, 213–220.

Itoh,N., Yonehara,S., Schreurs,J., Gorman,D.M., Maruyama,K., Ishii,A., Yahara,I., Arai,K. and Miyajima,A. (1990) *Science*, **247**, 324–327.

Kinashi,T. *et al.* (1986) *Nature*, **324**, 70–73.

Kitamura,T., Hayashida,K., Sakamaki,K., Yokota,T., Arai,K. and Miyajima,A. (1991a) *Proc. Natl. Acad. Sci. USA.*, **88**, 5082–5086.

Kitamura,T., Takaku,F. and Miyajima,A. (1991b) *Int. Immunol.*, **3**, 571–577.

Leonard,W.J. *et al.* (1984) *Nature*, **311**, 626–631.

Lopez,A.F., Eglinton,J.M., Gillis,D., Park,L.S., Clark,S. and Vadas,M.A. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 7022–7026.

Lopez,A.F., Eglinton,J.M., Lyons,A.B., Tapley,P.M., To,L.B., Park,L.S., Clark,S.C. and Vadas,M.A. (1990) *J. Cell Physiol.*, **145**, 69–77.

Mita,S., Harada,N., Naomi,S., Hitoshi,Y., Sakamoto,K., Akagi,M., Tominaga,A. and Takatsu,K. (1988) *J. Exp. Med.*, **168**, 863–878.

Mita,S., Tominaga,A., Hitoshi,Y., Sakamoto,K., Honjo,T., Akagi,M., Kikuchi,Y., Yamaguchi,N. and Takatsu,K. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 2311–2315.

Mita,S., Takaki,S., Hitoshi,Y., Rolink,A.G., Tominaga,A., Yamaguchi,N. and Takatsu,K. (1991) *Int. Immunol.*, **3**, 665–672.

Miyazaki,J., Takaki,S., Araki,K., Tashiro,F., Tominaga,A., Takatsu,K. and Yamamura,K. (1989) *Gene*, **79**, 269–277.

Murata,Y., Yamaguchi,N., Hitoshi,Y., Tominaga,A. and Takatsu,K. (1990) *Biochem. Biophys. Res. Commun.*, **173**, 1102–1108.

Nikaido,T., Shimizu,A., Ishida,N., Sabe,H., Teshigawara,K., Maeda,M., Uchiyama,T., Yodoi,J. and Honjo, T. (1984) *Nature*, **311**, 631–635.

Park,L.S., Friend,D., Price,V., Anderson,D., Singer,J., Prickett,K.S. and Urdal, D.L. (1989) *J. Biol. Chem.*, **264**, 5420–5427.

Rolink,A.G., Melchers,F. and Palacios,R. (1989) *J. Exp. Med.*, **169**, 1693–1701.

Sanderson,C.J., Campbell,H.D. and Young,I.G. (1988) *Immunol. Rev.*, **102**, 29–50.

Southern,P.J. and Berg,P. (1982) *J. Mol. Appl. Genet.*, **1**, 327–341.

Swain,S.L., McKenzie,D.T., Dutton,R.W., Tonkonogy,S.L. and English,M. (1988) *Immunol. Rev.*, **102**, 77–105.

Taga,T., Hibi,M., Hirata,Y., Yamasaki,K., Yasukawa,K., Matsuda,T., Hirano,T. and Kishimoto,T. (1989) *Cell*, **58**, 573–581.

Takaki,S., Tominaga,A., Hitoshi,Y., Mita,S., Sonoda,E., Yamaguchi,N. and Takatsu, K. (1990) *EMBO J.*, **9**, 4367–4374.

Takatsu,K., Tanaka,K., Tominaga,A., Kumahara,Y. and Hamaoka,T. (1980) *J. Immunol.*, **125**, 2646–2653.

Takatsu,K., Tominaga,A., Harada,N., Mita,S., Matsumoto,M., Takahashi,T., Kikuchi,Y. and Yamaguchi,N. (1988) *Immunol. Rev.*, **102**, 107–135.

Tominaga,A., Mita,S., Kikuchi,Y., Hitoshi,Y., Takatsu,K., Nishikawa,S. and Ogawa,M. (1989) *Growth Factors*, **1**, 135–146.

Tominaga,A., Takahashi,T., Kikuchi,Y., Mita,S., Naomi,S., Harada,N., Yamaguchi,N. and Takatsu,K. (1990) *J. Immunol.*, **144**, 1345–1352.

Tominaga,A. *et al.* (1991) *J. Exp. Med.*, **173**, 429–437.

Yamaguchi,N., Hitoshi,Y., Mita,S., Hosoya,Y., Murata,Y., Kikuchi,Y., Tominaga,A. and Takatsu,K. (1990) *Int. Immunol.*, **2**, 181–187.

Yamasaki,K., Taga,T., Hirata,Y., Yawata,H., Kawanishi,Y., Seed,B., Taniguchi,T., Hirano,T. and Kishimoto,T. (1988) *Science*, **241**, 825–828.

Yokota,T. *et al.* (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 7388–7392.

Yonehara,S., Ishii,A., Yonehara,M., Koyasu,S., Miyajima,A., Schreurs,J., Arai,K. and Yahara,I. (1990) *Int. Immunol.*, **2**, 143–150.

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