

Molecular characterization of the β chain of the murine interleukin 5 receptor

Seiji Mita, Satoshi Takaki, Yasumichi Hitoshi, Antonius G. Rolink¹, Akira Tominaga, Naoto Yamaguchi, and Kiyoshi Takatsu

Department of Biology, Institute for Medical Immunology, Kumamoto University Medical School, 2-2-1 Honjo, Kumamoto 860, Japan

¹Basel Institute for Immunology, Grenzacherstrasse, Basel, Switzerland

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Abstract

Interleukin 5 (IL-5) is a multifunctional cytokine that regulates the proliferation and differentiation of hematopoietic cells including B cells and eosinophils. The murine IL-5 acts on target cells via an IL-5 specific high-affinity receptor ($K_d = 150$ pM) that has been proposed to be composed of at least two membrane polypeptide chains. The p60 component recognized by anti-murine IL-5 receptor mAbs H7 and T21 binds IL-5 with low affinity ($K_d = 10$ nM). The other component is p130, detectable by following cross-linking experiments with IL-5. Using H7, T21, and R52.120 mAbs specific to murine IL-5 receptor, we characterized the molecular nature of the p130 of the high affinity receptor for murine IL-5. R52.120 mAb did not recognize the IL-5 binding recombinant p60 expressed on COS7 cells, but reacted with p130/140 on IL-5-dependent cell lines. R52.120 mAb showed partial inhibition of the IL-5-induced proliferation of the IL-5-dependent early B cell line Y16 at high IL-5 concentrations. Addition of R52.120 mAb together with H7 or T21 mAb caused more striking inhibition of the IL-5-dependent proliferation than that caused by either of them alone. R52.120 mAb down-regulated the number and dissociation constant of IL-5 binding sites with high affinity without affecting the levels of these with low-affinity. It also preferentially inhibited the formation of the cross-linked complex of p130 with radiolabeled IL-5. These results indicate that p130/p140, recognized by R52.120 mAb, is indispensable, together with p60, for the formation of high affinity IL-5 receptor. We propose to designate p60 and p130/p140 as the α and β chain of IL-5 receptor, respectively.

Introduction

Interleukin 5 (IL-5) is an inducible glycoprotein cytokine generated mainly by T cells following antigen or mitogen induction (1–3). The complementary DNAs encoding both mouse and human IL-5 have already been characterized (4–6). The growth and differentiation of B cells and other hematopoietic cells including eosinophils has been found to be regulated by IL-5 (7–12).

To elucidate how IL-5 mediates multiple functions in various target cells, we attempted to explore the molecular and biochemical characteristics of the receptor. On murine hematopoietic cells, both high and low affinity membrane IL-5 receptors (IL-5R) have been described with equilibrium dissociation constants (K_d) of ~ 150 pM and ~ 30 nM, respectively (13–16). Because most of the biological effects of IL-5 are observed at picomolar concentrations (13,14), it is clear that high-affinity IL-5R are biologically functional. Chemical cross-linking studies using [³⁵S]methionine labeled IL-5 ([³⁵S]IL-5) have suggested that high-affinity IL-5R is composed of at least two polypeptide chains

(14): one is the 60 kd ligand binding peptide (p60) recognized by H7 or T21 mAb (17,18) and the other is a putative membrane peptide of ~ 130 kd (p130). Both H7 and T21 mAbs completely inhibited the IL-5 binding to IL-5R under high affinity conditions. Rolink *et al.* (19) reported another series of mAbs (R52.120 and R52.625) that also recognize mouse IL-5R. It is not well characterized whether or not R52.120 mAb recognizes the p60 of IL-5 binding peptide.

We recently cloned the cDNA encoding p60 of murine IL-5R utilizing a high-efficiency COS7 cell expression cloning system using H7 and T21 mAbs-mediated cell panning procedures (20). The cDNA transfection experiments in COS7 cells have shown that p60 can be individually expressed and is capable of binding IL-5 with low affinity ($K_d = 10$ nM) by itself. When FDC-P1 cells (IL-3-dependent myeloid cell line) (20) were transfected with the IL-5R cDNA, they expressed IL-5R with both high ($K_d = 30$ pM) and low affinity ($K_d = 6$ nM) and were responsive to IL-5,

Correspondence to: K. Takatsu

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though parental FDC-P1 cells could neither bind IL-5 nor respond to IL-5. So, we considered that an additional molecule(s) is present which may not bind IL-5 by itself and that may be required to constitute high affinity IL-5R and play an important role in the IL-5 signal transduction.

We describe herein the characterization of the molecular components of murine high-affinity IL-5R, namely p130, utilizing H7, T21, and R52.120 (R52) mAbs. It will be shown that R52 mAb immunoprecipitates a p130/p140 membrane peptide from the IL-5 dependent early B cell line, Y16, but does not recognize p60 on COS7 cells transfected with the murine IL-5R cDNA. R52 mAb down-regulates the number and affinity of high-affinity IL-5R. The results will support the notion that p130/p140 recognized by R52 mAb is an indispensable component of the high affinity IL-5R together with the p60 component, although it does not bind IL-5 by itself. We propose to designate p60 and p130/p140 as the α and β chain of IL-5R, respectively.

Methods

Cytokines

Recombinant mouse IL-5 (rIL-5, 2.2×10^{10} units/g protein) and [35 S]IL-5 were prepared according to procedures previously described (2,13). The specific activities of [35 S]IL-5 were $4.0 - 6.2 \times 10^{15}$ c.p.m./mmol. Mouse rIL-3 was kindly provided by Dr T. Sudo (Biomedical Research Center, Kamakura, Japan).

Cell lines

In vitro cell lines were maintained in RPMI 1640 medium supplemented with heat-inactivated 5% FCS (Cell Culture Technologies, Inc., Toronto, Canada), $50 \mu\text{M}$ 2-ME, $100 \mu\text{g/ml}$ streptomycin and 100 U/ml penicillin. IL-5-dependent early B cell lines, Y16 and T88-M, were established by long-term bone marrow culture and maintained *in vitro* in the presence of IL-5 as described (12,20). Both IL-3-dependent IC2 (21) (kindly provided by Dr shin Yonehara, Tokyo Metropolitan Institute of Medical Science) and FDC-P1 cells (22) (kindly provided by Dr Kiyoshi Sakamoto, the Second Department of Surgery, Kumamoto University Hospital, Kumamoto, Japan) were maintained in the presence of 10 units/ml IL-3.

Expression of the IL-5 receptor cDNA

The cDNA encoding the murine IL-5 receptor was transfected into COS7 cells as described (20). In brief, the cDNA of pIL-5R.8 was inserted into pCAGGS, a derivative of pAGS-3 (23) (kindly provided by Dr Jun-Ichi Miyazaki, Kumamoto University Medical School) using an *Xho*I linker, resulting in pCAGGS-5R. COS7 cells were transfected with pCAGGS-5R by the DEAE-dextran method, trypsinized at 8 h post-transfection and re-seeded in Petri dishes. After 2 or 3 days of incubation, cells were harvested after a brief treatment with 0.5 mM EDTA and subjected to further analysis.

Monoclonal antibodies

H7 (IgG_{2a}), T21 (IgG1) and R52.120 (R52) (IgG1) anti-murine IL-5R mAbs were prepared and purified from the ascites of H7- and T21-bearing BALB/c-nu/nu mice and from cultured supernatants of R52.120 cells by using Protein G Sepharose (Pharmacia, Uppsala, Sweden) (17,19).

Flow cytometry

Cells (1×10^6) were stained with R52 or H7 mAb and FITC-coupled F(ab')₂ fragments of goat anti-rat IgG (Cappel Laboratories, Malvern, PA). Negative controls were cells incubated with FITC-coupled F(ab')₂ fragments of goat anti-rat IgG alone. These populations were then analyzed on FACScan (Becton-Dickinson, Sunnyvale, CA).

Binding assay

Binding assay was performed according to the protocol described previously using biosynthetically labeled [35 S]IL-5 (13). To assess equilibrium radiolabeled IL-5 binding, $1 - 10 \times 10^6$ Y16 cells were incubated at 37°C for 10 min with various concentrations of radiolabeled IL-5. At the end of incubation, the cell-associated radioactivity was counted. The specific binding was defined as the difference between total binding and nonspecific binding in the presence of 100-fold molar excess of unlabeled IL-5. The dissociation constant (K_d) and an average number of binding sites per cell were calculated by Scatchard plot analysis of the saturation binding data (24).

Chemical cross-linking of IL-5 binding proteins

Disuccinimidyl tartarate (DST) was purchased from Pierce Chemical Co. (Rockford, IL) [35 S]IL-5 was cross-linked to IL-5 binding proteins as previously described (14). In brief, Y16 cells (10^7 cells) in $200 \mu\text{l}$ of binding medium in the presence or absence of 100-fold excess unlabeled IL-5 were incubated with [35 S]IL-5 (100 pM) for 10 min at 37°C . After extensive washing, DST (50 mM) in DMSO was added to give a final concentration of 1 mM , and the mixture was incubated for 30 min on ice. The cells were then washed twice with HBSS and resuspended in $300 \mu\text{l}$ of 'lysis buffer' (pH 7.2) (PBS, 1% Triton X-100, 2 mM EGTA, 2 mM EDTA, 2 mM PMSF, $10 \mu\text{M}$ pepstatin, $10 \mu\text{M}$ leupeptin, 2 mM *O*-phenanthroline, 200 KIU/ml aprotinin). The cell lysates were subjected to SDS-PAGE using the stacking gel procedure of Laemmli (25) and analyzed by fluorography (14). Molecular standards (Bio-Rad Laboratories) were myosin (200 kd), β -galactosidase (116 kd), phosphorylase b (97.4 kd), BSA (66.2 kd), ovalbumin (42.7 kd), and carbonic anhydrase (31 kd).

Immunoprecipitation of membrane protein by mAbs

Cells ($2 - 6 \times 10^8$) were radioiodinated using Iodo-Beads (Pierce) (20). Radiolabeled cells were lysed with 'lysis buffer' (pH 7.2). Detergent extracts were preadsorbed with $50 \mu\text{l}$ of Protein G-coupled Sepharose (Pharmacia) for 1 h at room temperature. Then resulting samples were incubated with H7 or R52 mAbs followed by the incubation with Protein G-coupled Sepharose beads at 4°C for 12 h. The immunoprecipitates on the beads were washed three times at 4°C with washing buffer containing 1% Triton X-100, 0.1% SDS, 0.15 M NaCl, and 50 mM HEPES (pH 7.2). The beads were boiled in $30 \mu\text{l}$ of SDS sample buffer for 3 min. Samples thus obtained were subjected to SDS-PAGE according to the method described by Laemmli (25) using 7.5% polyacrylamide gels with slight modifications and analyzed by autoradiography.

Proliferation assay

IL-5-induced proliferation of Y16 cells was assessed by incorporation of [methyl- ^3H]thymidine (sp. act., 2 Ci/mmol ; New

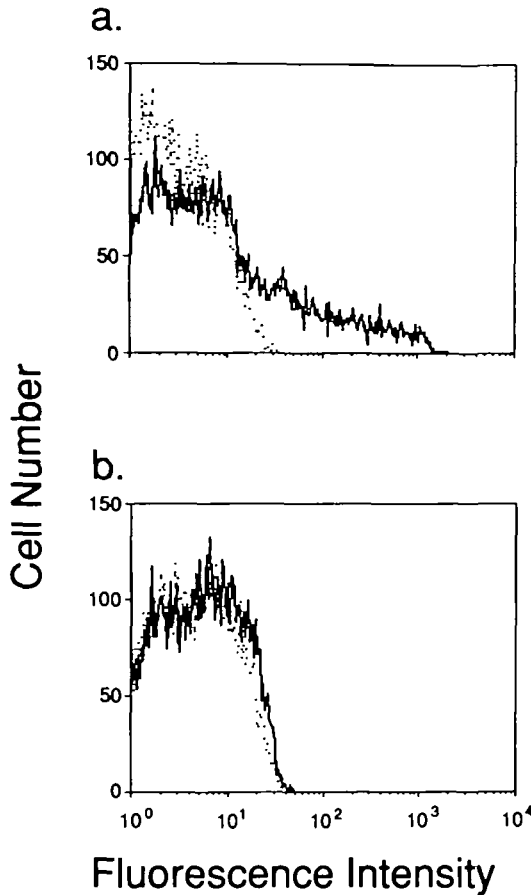


Fig. 1. Flow cytometry analysis of COS7 cells transfected with murine IL-5R cDNA. COS7 cells transfected with the IL-5R cDNA (pCAGGS-5R) (—) or transfected with control vector (pCAGGS) (----) were stained with either H7 (a) or R52 mAb (b) in combination with 50 μ l FITC-coupled F(ab')₂ fragments of goat anti-rat IgG (2.5 μ g/ml). The stained cells were analyzed with a FACScan flow cytometer. Relative fluorescence intensity was expressed by logarithmic amplification.

England Nuclear). In brief, Y16 cells ($5 \times 10^4/0.2$ ml/well) were cultured in a microplate in the presence of various concentrations of IL-5 and pulsed with 0.2 μ Ci/ μ l of [³H]thymidine during the last 12 h of a 36 h culture. DNA synthesis was determined by means of [³H]thymidine incorporation.

Results

R52 mAb recognizes a different molecule (p130/p140) from p60 of the murine IL-5R

As we reported (17,18), H7 and T21 mAbs recognize p60 of IL-5 binding molecule of IL-5R complex. Rolink *et al.* (19) reported that R52 mAb recognizes p46, p130, and p140. To evaluate whether R52 mAb can recognize molecule(s) identical to that recognized by H7 mAb, we transfected COS7 cells with murine IL-5R cDNA (pCAGGS-5R) and stained transfectants with R52 or H7 mAb in combination with FITC-coupled F(ab')₂ fragments of goat anti-rat IgG and analyzed by flow cytometry. The COS7 transfectants expressed $\sim 8 \times 10^5$ IL-5 binding sites per cell with an apparent K_d of 9.6 nM. H7 mAb immunoprecipitated p60 from lysates of ¹²⁵I-labeled COS7 cells as described (20). As shown in Fig. 1, COS7 transfectants displayed positive staining with H7 (a), but not with R52 mAb (b), indicating that R52 mAb does not recognize the IL-5 binding recombinant p60 protein. We then stained 12 IL-5-dependent cell lines with R52 or H7 mAb. We also examined the reactivity of the mAbs with IL-3-dependent cell lines, IL-2-dependent CTLL2, and L cells. As shown in Fig. 2, all 12 of the IL-5-dependent cell lines represented by Y16 and T88-M reacted with both R52 and H7 mAbs. Neither IL-2-dependent CTLL2 nor L cells showed positive staining to both mAbs. It is of particular interest that IL-3-dependent cell lines represented by FDC-P1 and IC2 were stained with R52 mAb, but not H7 mAb.

To confirm that R52 mAb recognizes molecule(s) different from p60, immunoprecipitation experiments were carried out. When Y16 cells were radioiodinated and cell extracts were immuno-

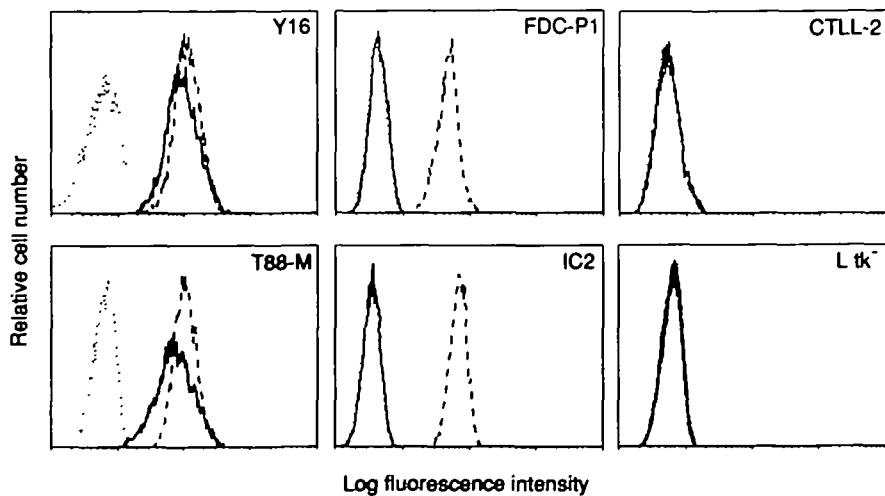


Fig. 2. Flow cytometry analysis of cloned cell lines. Various cloned cell lines were stained with 0.5 μ g/50 μ l of either H7 (—) or R52 mAb (---) in combination with FITC-coupled F(ab')₂ fragments of goat anti-rat IgG as described in Fig. 1. As negative controls (· · · · ·), cells were stained with FITC-coupled F(ab')₂ fragments of goat anti-rat IgG. The stained cells were analyzed with a FACScan flow cytometer. Relative fluorescence intensity was expressed by logarithmic amplification.

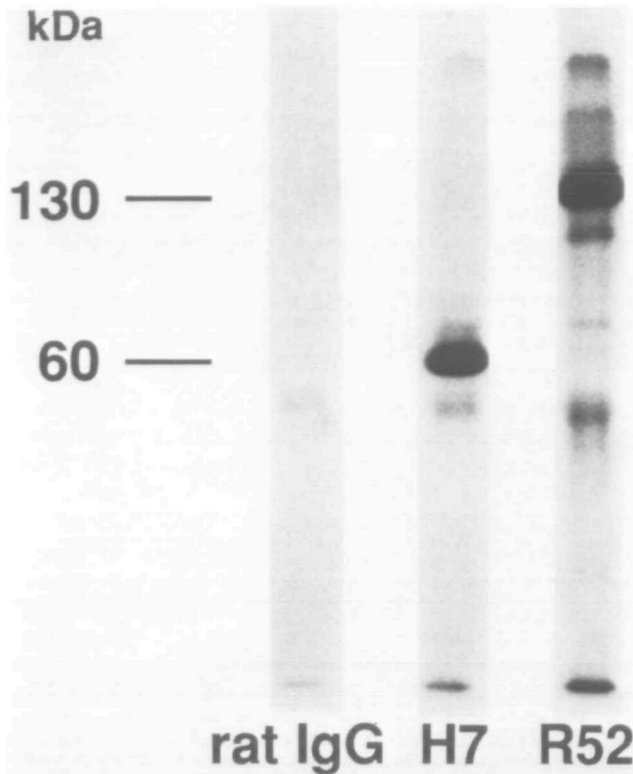


Fig. 3. SDS-PAGE and autoradiography of immunoprecipitation using H7 or R52 mAbs. Y16 cells (2×10^7) were radiolabeled and membrane-fractions were prepared. Membrane-fractions were immunoprecipitated using H7 or R52 mAb. The immunoprecipitated samples were analyzed using SDS-PAGE under reducing conditions

precipitated by R52 mAb, two bands of p130 and p140 were observed on SDS-PAGE (Fig. 3). Although the results were in agreement with those reported by Rolink *et al.* (19), these were in sharp contrast to the detection of a single major band of p60 when lysates were immunoprecipitated with H7 mAb. These results clearly indicate that R52 mAb recognizes membrane proteins p130 and p140 (p130/p140) that are not recognized by H7 or T21 mAbs. As a minor band, a p120 band was also detected, although it is not clear whether it is a degradation product of major immunoprecipitates. It is also unclear whether or not p130 is identical to p140.

Synergistic inhibitory effect of R52 and H7 mAb on IL-5-induced proliferation of Y16 cells

Having obtained the data described above, we anticipated that R52 mAb would inhibit the IL-5-dependent growth of Y16 cells in a manner different from H7 or T21 mAbs. Y16 cells were cultured with a wide range of IL-5 concentrations (0.01–10 pM) in the presence of R52 mAb (10 μ g/ml; a sufficient concentration to abrogate more than 20% of [35 S]IL-5 binding at 100 pM) or in the presence of H7 mAb (10 μ g/ml; a sufficient concentration to compete with 1 nM [35 S]IL-5 for binding to the p60 alone), or in combination with R52 and H7 mAbs. As shown in Fig. 4, Y16 cells incorporated [3 H]thymidine in response to IL-5 in a dose-dependent manner and its incorporation reached a plateau

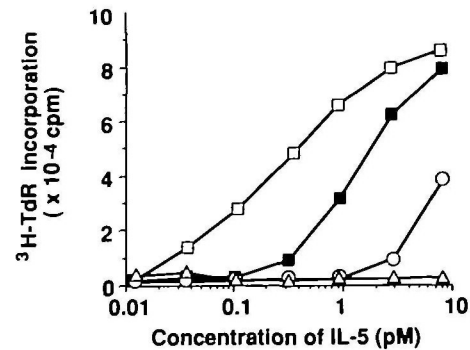


Fig. 4. Inhibitory effects of mAbs on IL-5-induced DNA synthesis of IL-5-dependent Y16 cells. Y16 cells ($5 \times 10^4/0.2$ ml) were distributed into a microplate (0.2 ml/well) together with various concentrations of IL-5. The mAbs to be tested were simultaneously added at a concentration of 10 μ g/ml in triplicate cultures and cells were cultured for 36 h at 37°C. Data were obtained with concentrations of purified antibodies, H7 (O), R52 (■), H7 plus R52 (Δ), and normal rat IgG (□). Cells were pulsed with 0.2 μ Ci [3 H]thymidine for the last 12 h of culture. Each point expresses mean c.p.m. of triplicate cultures. One of the representative results of three different series of experiments was shown. Y16 cells incorporated 428 c.p.m. in the absence of IL-5

beyond 5 pM IL-5. Addition of R52 mAb partially inhibited DNA synthesis of Y16 cells at IL-5 concentrations higher than 0.3 pM, although it completely inhibited the DNA synthesis at lower IL-5 concentrations. In contrast, H7 mAb almost completely inhibited the IL-5-induced proliferation even at 3 pM IL-5 and inhibited by more than 60% at 9 pM IL-5. Similar results to the above were obtained even when 10-fold higher concentrations of each of mAb (100 μ g/ml) were used (data not shown). Intriguingly, simultaneous addition of both R52 and H7 mAbs caused the cooperative effects resulting in the striking suppression of proliferative response of Y16 cells induced by IL-5 even at 10 pM. The addition of T21 mAb (10 μ g/ml) caused almost complete inhibition of the proliferative response of Y16 cells induced by 10 pM IL-5 (data not shown) that may be due to a higher affinity of T21 mAb than that of H7 mAb to its receptor (18). The inhibitory effect of T21 mAb decreased, however, along with the addition of increased concentrations of IL-5 into the culture of Y16 cells (Fig. 5a) irrespective of the amounts of mAb added. It is of particular interest that simultaneous addition of both T21 and R52 mAbs again caused enhanced inhibition on the proliferation induced by 1–5 nM IL-5 (Fig. 5b). We also obtained essentially identical results using T88-M cells in place of Y16 cells. These results strongly suggest that R52 mAb may show a different mode of inhibition of IL-5-induced proliferation from that of H7 mAb, besides a much more weak inhibitory activity than that of H7 mAb.

R52 mAb preferentially down-regulates the number of high-affinity IL-5 binding sites

To further evaluate the role of p130/p140 in the high-affinity IL-5R complex, an IL-5 binding study was carried out in the presence or absence of R52 mAb using Y16 cells. As shown in Table 1, R52 mAb (10 μ g/ml) caused only partial inhibition (up to 20%) of the binding of [35 S]IL-5 (100 pM) to Y16 cells, and the addition of excess amounts of R52 mAb (30 μ g/ml) did not change the extent of the inhibition (data not shown). Both H7 mAb and excess amounts of unlabeled IL-5 also completely

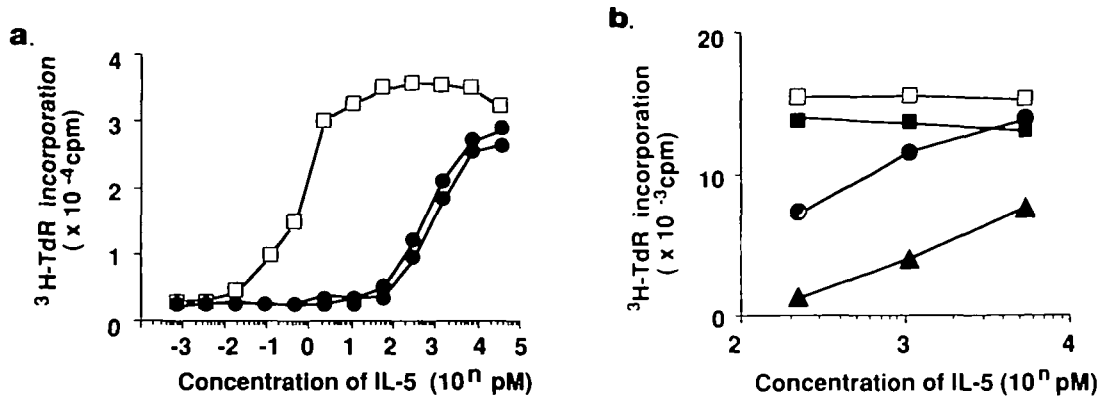


Fig. 5. Inhibitory effects of mAbs on IL-5-induced DNA synthesis of IL-5-dependent Y16 cells. Y16 cells were cultured with various concentrations of IL-5 as described in Fig. 4. (a) Either 10 (●) or 100 $\mu\text{g/ml}$ (●) of T21 mAb was simultaneously added on day 0, or (b) R52 mAb (10 $\mu\text{g/ml}$) (■), T21 mAb (10 $\mu\text{g/ml}$) (●), both R52 and T21 mAbs (▲), or control IgG (□) was added at the onset of the culture. Cells were cultured for 36 h at 37°C. Cells were pulsed with 0.2 μCi [^3H]thymidine for the last 12 h of culture. Each point expresses mean c.p.m. of triplicate cultures. One of the representative results of three different series of experiments was shown. Y16 cells incorporated 468 c.p.m. in the absence of IL-5.

inhibited the IL-5 binding to Y16 cells. Scatchard plot analysis of the saturated binding data showed that Y16 cells consistently showed two IL-5 binding sites (high affinity, K_d 60 pM, ~1200 sites per cell; low affinity, K_d 21 nM, ~15,000 sites per cell) (Fig. 6). When we carried out the binding assay in the presence of mAb, the number of high-affinity binding sites were significantly reduced by R52 mAb (~600 binding sites per cell) and the affinity decreased to a K_d of ~200 pM. In contrast, the number and K_d of low-affinity IL-5 binding sites was not significantly affected by R52 mAb. Both high and low affinity IL-5 binding sites were hardly detected in the presence of either H7 or T21 mAb under the same conditions employed (data not shown).

We have reported in our previous cross-linking studies that the p130 protein is involved in the formation of a 160 kd cross-linked complex with IL-5, while the p60 protein is involved in the formation of both 100 kd and 160 kd cross-linked complexes (14,17). The 160 kd cross-linked complex is detectable on IL-5-responsive cells under high affinity conditions (14). To examine the effect of R52 mAb on chemical cross-linking, the IL-5 binding protein on the cell surface of Y16 cells was cross-linked to [^{35}S]IL-5 under high affinity conditions (100 pM [^{35}S]IL-5) in the presence or absence of mAbs. As can be seen in Fig. 7, two major cross-linked bands of ~100 and 160 kd were identified in the absence of mAb. Neither band could be detected if unlabeled and ^{35}S -labeled IL-5 were added together (data not shown). Only the 100 kd cross-linked band was detected when R52 mAb was added before cross-linking, while neither one of the cross-linked complexes was detected when H7 or T21 mAbs were added before cross-linking.

Discussion

The existence of both low-affinity ($K_d \approx 30$ nM) and high-affinity ($K_d \approx 150$ pM) forms of murine IL-5R on early B cells and eosinophils has raised the question of the structural relationship of one to the other. We have proposed that the murine high-affinity IL-5R is a membrane complex composed of at least two polypeptide chains: p60 and p130. We also reported, using H7 and T21 mAbs, that the p60 component appears to be the IL-5

Table 1. Inhibitory effects of mAbs on the binding of radiolabeled IL-5 to Y16 cells

Inhibitors	Specific binding (c.p.m.)
None	1396
IL-5 (1 nM)	128
H7 (10 $\mu\text{g/ml}$)	159
R52 (10 $\mu\text{g/ml}$)	1068

Y16 cells ($5 \times 10^5/\text{ml}$) were cultured for 2 h at 37°C and then pretreated with IL-5 or mAb inhibitor at 4°C for another 2 h before the binding assay using 100 pM [^{35}S]IL-5

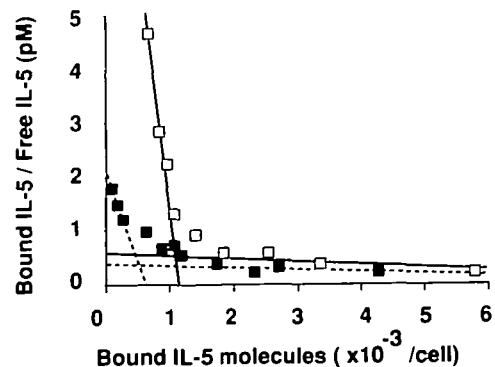


Fig. 6. Scatchard plot of equilibrium binding analysis of [^{35}S]IL-5 to Y16 cells. Cells (1×10^6) were incubated at 37°C with various concentrations of [^{35}S]IL-5 for 10 min. Specific equilibrium binding was determined after subtraction of nonspecific binding obtained in the presence of a 100-fold molar excess of unlabelled IL-5. Saturation binding data were re-expressed as a Scatchard plot. The binding assay was carried out in the absence (□) or in the presence (■) or H7 (●) mAb (10 $\mu\text{g/ml}$).

binding molecule (17,18). This was finally demonstrated by isolation of the cDNA encoding p60 that can bind IL-5 with low-affinity ($K_d \approx 10$ nM) (20). Although the identification of p60 has been

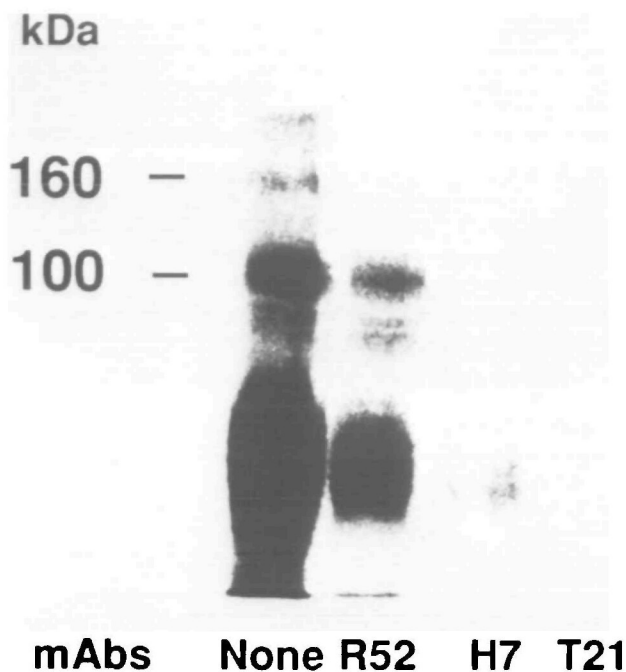


Fig. 7. Fluorograph of chemical cross-linking of [^{35}S]IL-5 binding protein. Y16 cells (2×10^7) were incubated with [^{35}S]IL-5 (0.3 nM, 2×10^5 c.p.m.) for 10 min at 37°C in the presence or in the absence of mAbs (10 $\mu\text{g/ml}$). The cells were then washed, and DST (50 mM) was added and kept at 4°C for another 30 min. After several washings, cells were lysed with the buffer as described in Methods and the detergent lysates were analyzed on SDS-PAGE with 7.5% acrylamide gel under nonreducing conditions. Fluorography was performed after the electrophoresis. The uncross-linked IL-5 ran at the middle of the 7.5% gels, which was ~ 50 kd.

definitively made, the existence of p130 has only been suggested by cross-linking studies using radiolabeled IL-5 (14). The major findings observed in this study are the following. (i) R52 mAb against murine IL-5R does not react with the murine IL-5 binding recombinant p60 protein (Fig. 1), but reacts with all IL-5-responsive cell lines so far tested (Fig. 2). (ii) R52 mAb immunoprecipitates p130/p140 doublets from cell lysates of IL-5-dependent Y16 cells (Fig. 3). It is not clear at this moment, however, whether or not p130 is an identical protein to p140 with a slight difference in carbohydrate content. (iii) R52 and H7 mAbs show a little and partial inhibition, respectively, of IL-5-induced proliferation of Y16 cells at high IL-5 concentrations (Fig. 4). However, simultaneous addition of R52 mAb together with H7 mAbs causes higher synergistic inhibition of IL-5-induced proliferation than that caused by the addition of each one of them separately. (iv) R52 mAb partially inhibits IL-5 binding to target cells under high affinity conditions (100 pM [^{35}S]IL-5) while H7 or T21 mAbs profoundly inhibit the binding (Table 1). Furthermore, R52 mAb preferentially down-regulates the number and K_d of high affinity IL-5-binding sites (Fig. 6). (v) R52 mAb inhibits the formation of a cross-linked complex between p130/p140 and IL-5 (Fig. 7). Taking all of the results together, p130/p140 recognized by R52 mAb appears to be the most likely candidate for the second chain or 'associated' subunit to form high affinity IL-5R together with the IL-5 binding p60 protein.

There are at least three possibilities to account for the effects of R52 mAb on the IL-5-induced proliferation and down-regulation of the number and K_d of high-affinity IL-5R. If we assume that IL-5 first binds to p60 and that a complex of p60 and IL-5 then associates with p130/p140 resulting in the formation of high affinity receptor complex, similar to a converted model of the IL-2R system (26), the first possibility is that p130/p140 may bind IL-5 in conjunction with p60, and R52 mAb may inhibit the association of p130/p140 with IL-5. Another possibility is that p130/p140 may associate with epitopes on p60 other than the IL-5-binding epitope. In that case, R52 mAb may recognize epitope(s) with identical or close proximity to the association sites of p130/p140 with p60 and inhibit this kind of association by steric hindrance. The third possibility is that R52 mAb recognizes an epitope which is unrelated to the association sites with IL-5 or p60 and that the binding of R52 mAb to p130/p140 may cause conformational changes of the p130/p140 molecule leading to unsuccessful interaction of p130/p140 with a complex of IL-5 and/or p60. We are in favor of the second possibility on the basis of the following findings. R52 mAb could inhibit the formation of the 160 kd cross-linked complex (Fig. 7). Furthermore, immunoprecipitation experiments of chemically cross-linked complex revealed that R52 mAb could immunoprecipitate the 160 kd cross-linked complex (data not shown).

There are several reports to support the notion that functional receptors for cytokines consist of two different polypeptide chains, namely α and β subunits. In the case of IL-2R system, the α chain (p55) binds IL-2 with low-affinity (27) and the β chain (p75) by itself also binds IL-2 with intermediate affinity (28). Co-expression of both α and β chains leads to the formation of a high-affinity receptor, and both chains can be cross-linked with IL-2 (28). In the case of the IL-6R system, transfection of the cDNA encoding the IL-6 binding molecule in COS cells can induce the expression of mainly low-affinity binding sites (29). IL-6 triggers the association of IL-6R and non-ligand binding membrane glycoprotein, gp130, and generates IL-6 signal (30). Soluble IL-6R and IL-6 can also interact with cell surface gp130. Hibi *et al.* (31) recently cloned the gene encoding gp130 that does not show a binding property to IL-6 by itself. A cloned gp130 can associate with a complex of IL-6 and IL-6R. Hayashida *et al.* (32) recently reported that the low-affinity human GM-CSFR, together with the KH97 protein encoded by a human cDNA homologous to murine IL-3R cDNA, forms a high-affinity receptor for human GM-CSFR, although KH97 protein is able to bind neither human GM-CSF nor IL-3. Because of similarities among the IL-2, GM-CSF, and IL-5 high-affinity receptors, we propose to designate the low-affinity mouse IL-5R as the α chain and the R52 protein (p130) as the β chain of mouse IL-5R.

After transfection of COS7 cells with a cDNA encoding p60, cell surface murine IL-5R were expressed at a density of $10^5 - 10^6$ per cell as a single binding class of low-affinity (K_d 6–9 nM). Despite careful analysis, no high-affinity IL-5-binding was detected (20). It is of particular interest that FDC-5R cells, established from IL-3-dependent FDC-P1 cells by transfection with murine IL-5R cDNA, express ~ 500 binding sites per cell for IL-5 with an apparent K_d of 30 pM (high-affinity) and 8000 binding sites per cell with K_d of 6 nM (low affinity), and become IL-5 responsive for DNA synthesis (20). We have therefore postulated that the IL-5 binding p60 binds to IL-5 with low affinity and can be converted to a high-affinity IL-5R by interaction with

the second chain or 'associated' subunit (p130) that is expressed in FDC-P1 cells. As shown in Fig. 2, FDC-P1 and IC2 cells react with R52 but not with H7 mAb. These IL-3-dependent cell lines neither bound IL-5 determined by the IL-5-binding assay nor responded to IL-5 (data not shown). This may support the notion that the p130/p140 protein is the *β* chain of IL-5R.

Rolink *et al.* (19) reported that R52 mAb inhibits the IL-5-driven proliferation of B₁₃ cells and the binding of IL-5 to the cells. They also reported that high concentrations of R52 mAb inhibits the IL-3-driven proliferative response of B₁₃ cells by 50% but does not affect the other IL-3-sensitive cell lines and speculated the connection of the IL-3 and IL-5 responsiveness and of the corresponding receptors on B₁₃ (19). We also observed the partial inhibition by R52 mAb on IL-3-induced proliferation of Y16 and T88-M cells (data not shown). Furthermore, we recently detected similar patterns of protein phosphorylations at serine and tyrosine residues of cellular proteins when T88-M cells were stimulated either with IL-5 or IL-3 (33). Thus, we speculate the presence of interaction between the IL-5R and IL-3R systems via p130/p140.

In summary, we demonstrate that murine high affinity IL-5R is a complex of two polypeptides composed of the IL-5 binding p60 protein and the p130/p140 that has no IL-5 binding ability by itself. In any case, identification of p130/p140 of R52 protein is urgently required to clarify the molecular nature of the second chain of high-affinity IL-5R that may be involved in the IL-5 signal transduction. The experimental system described in this study will give us new insights for elucidating IL-5 and the IL-5R system.

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Abbreviations

DST	disuccinimidyl tartarate
IL	interleukin
IL-5R	IL-5 receptor
H7	rat mAb against murine IL-5 receptor
R52	rat mAb against murine IL-5 receptor

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