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# Antibodies to rat soluble IL-6 receptor stimulate B9 hybridoma cell proliferation

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**Abstract Interleukin-6 mediates its pleiotropic effects by interacting with its membrane bound receptor (gp80) or the soluble counterpart gp54, resulting in activation of a complex that includes the transducer protein gp130. We have generated a polyclonal antibody against the rat soluble IL-6 receptor (anti-rat sIL-6R) in rabbits. By Western blot analysis we show that purified anti-rat sIL-6R IgG antibody reacts specifically with recombinant rat sIL-6R generated from *E. coli*, baculovirus or adenovirus expression systems. Anti-rat sIL-6R inhibited IL-6-induced acute phase protein synthesis in rat (H35) but not human (HepG2) hepatoma cells, and did not affect stimulation of those cells by Oncostatin-M. Conversely, on the mouse hybridoma B9 cell line, IgG anti-rat sIL-6R showed a dose-dependent stimulation of proliferation. Fab fragments of this antibody did not stimulate, but abrogated IL-6-mediated hepatoma cell stimulation and B9 cell proliferation. Gel shift analysis of STAT nuclear factors showed activation of STAT DNA binding in nuclei of B9 cells treated with IgG anti-rat sIL-6R, whereas in H35, NIH-3T3 and M1 cells, only IL-6 could trigger a similar STAT activation. Our data suggest that mechanisms of IL-6 receptor activation and signalling in mouse B9 hybridoma cells show subtle but important differences from other IL-6-responsive cells.**



*Key words:* IL-6 receptor; B9 hybridoma; Signalling; STAT

## 1. Introduction

Interleukin-6 (IL-6) belongs to the family of hematopoietic cytokines that includes IL-6, Interleukin-11 (IL-11), Oncostatin-M (OSM), leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF) and cardiotrophin-1 (CT-1) (for review see [1,2]). These cytokines share various activities since they act through receptor complexes that include the common signal transducer glycoprotein, gp130. The IL-6 receptor complex includes the unique ligand-binding IL-6 receptor chain (IL-6R). IL-6 interacts first with membrane bound IL-6R or its soluble counterpart (sIL-6R), and this primary complex interacts with gp130 [3,4]. IL-6-induced dimerization of gp130 activates Janus kinase (Jak) family of tyrosine kinases, depending upon the cell types (for review see [5,6]), which phosphorylate several tyrosine residues along gp130. This allows recruitment, through the SH2 domain, of members of the signal transducers and activators of transcription (STAT) family, namely STAT-1 and STAT-3, which also become phosphorylated by the Jak bound to gp130 [7,8]. Activated STAT proteins then form hetero- and homo-dimers and translocate to the nucleus where they behave as transcriptional factors and bind to specific promoter sequences (such as APR-RE and NF-IL-6RE) upstream of IL-6-induced genes such as acute phase proteins in the liver [9,10].

The presence of the soluble IL-6 receptor (extracellular domain) has been shown to enhance responses to IL-6 in hepatocytes [11,12] and in other cells that possess gp130 but lack the membrane IL-6R form [13–16]. This is in contrast to the action of other soluble receptors which inhibit ligand function [17,18]. In order to specifically inhibit biological responses mediated by the IL-6 receptor in rat cells in vitro and in vivo, we generated specific antibodies to the rat IL-6 soluble receptor. In this paper we show that purified polyclonal antibodies against rat sIL-6R can specifically inhibit IL-6-induced acute phase protein stimulation in hepatocytes. The antibody also inhibits IL-6-induced STAT activation in rat hepatocytes and in other IL-6-responsive cells (mouse M1 and NIH-3T3 cells). In contrast, the antibodies possess a potent agonistic activity on proliferation of the mouse hybridoma B9 cell line, and can activate STAT proteins in B9 cells. Purified specific Fab fragments on the other hand inhibit B9 cell proliferation and associated STAT activation. These studies have raised several issues regarding qualitative differences between B9 cells and other IL-6-responsive cells within these model systems.

## 2. Material and methods

### 2.1. Acute phase protein production and B9 cell proliferation

The effects of cytokines or antibody on human HepG2 and rat H35 hepatoma cell production of acute phase proteins was determined on confluent monolayer cells in 24 well cluster plate containing 300 μl of media. Cells were stimulated for 48 h, media was removed and the concentrations of acute phase proteins were analyzed by rocket immunoelectrophoresis using agarose containing specific antibody as previously described [19]. The B9 hybridoma proliferation assay was performed as previously described and analyzed using a colorimetric development [20,21].

### 2.2. Antibody and cytokines

Specific antibodies against the rat soluble IL-6 receptor were generated in rabbits immunized with a replication deficient recombinant adenovirus encoding the soluble form of the rat IL-6R as previously described (Thibault et al., manuscript submitted). Fab fragments were generated using immobilized papain (Pierce Biochemicals) according to manufacturer's instructions. Fc fragments were removed by chromatography on a protein A column (Pharmacia). Rat IL-6 was produced in *E. coli* and affinity purified on a Ni chelate column as previously described [22]. Human IL-6 and OSM are generous gifts from Dr. M. Widmer (Immunex Corp., Seattle, WA) and Dr. P. Wallace (Bristol-Myers Squibb Research Institute, Seattle, WA), respectively.

### 2.3. Gel shift assay

Cells were treated with cytokines and/or antibody as indicated for 15 min. Nuclear extracts were prepared as described previously [23]. Oligo nucleotide probes corresponding to the high affinity sis-inducible element SIE [24] were annealed, labeled and purified (sequences are: 5′-GTCGACATTTCCCGTAAATC-3′ and 5′-TCGACGATT-

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TACGGGAAATG-3′). For the assay, nuclear extracts were incubated for 15 min on ice with 2 μg of poly (dI.dC) and 5 μg of calf thymus DNA in binding buffer as described. Probe ($2\times10^5$ cpm) was added and the reaction was incubated 20 min at r.t. Loading buffer was added, and samples were electrophoresed (5% polyacrylamide) at 95 V for 4 h. The gels were then dried before autoradiography.

*2.4. Western blot*

Proteins were run on a 7.5 or 10% polyacrylamide gel under reducing conditions. The gels were transferred onto a nitrocellulose membrane (Immobilon). After blocking for 1 h into 5% blotto, 1% BSA, Tween 1% PBS, the antibodies were added to a dilution of 1/5000 in the same solution. Development was carried out with a secondary antibody (goat anti-rabbit, SIGMA) labeled with peroxidase and a chemiluminescence system (Dupont-NEN) according to manufacturers specification.

## 3. Results

*3.1. Antibodies raised against rat sIL-6R react specifically with recombinant rat IL-6R produced in E. coli, insect cells or mammalian cells*

Specific antibodies were raised in rabbits by vaccination with a recombinant adenovirus expressing the extracellular domain of rat IL-6R (Thibault et al., manuscript submitted) and boosting with baculovirus expressed purified recombinant sIL-6R protein. A Western blot using this antibody shows specific reactivity with rat sIL-6 expressed as a 33 kDa protein from *E. coli* (lane 1), with 50 kDa rat sIL-6R expressed in baculovirus [19] (lane 2) and with a 55 kDa protein expressed from adenovirus vector in infected human HepG2 (lane 3) or rat H35 hepatoma cells (lane 5) (Fig. 1). Lanes 4 and 6 show supernatants of HepG2 or H35 cell supernatants infected with control virus and indicate no reactivity. The difference in size of materials detected is likely due to the lack of glycosylation in prokaryotic cells and different glycosylation patterns between insect and mammalian cells. The antibody we used had been purified (IgG fraction) and has been termed anti-rat sIL-6R.

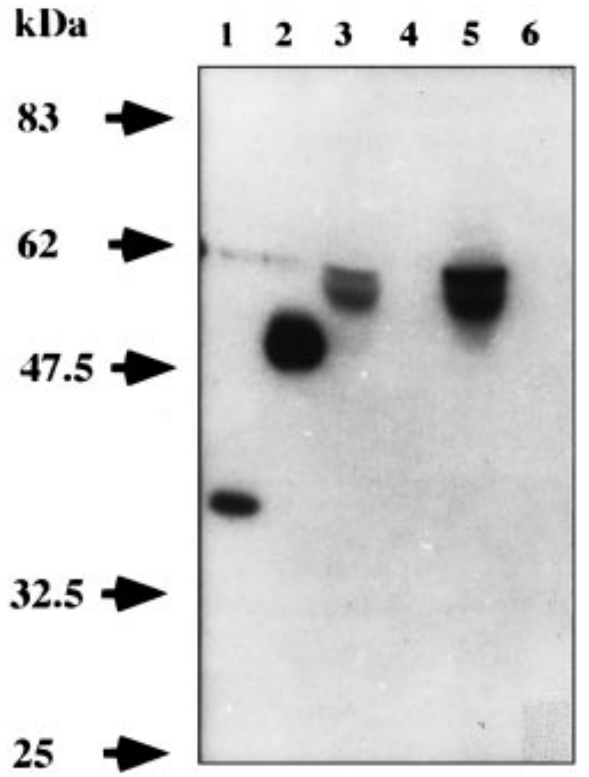


Fig. 1. Specific IgG recognize different preparations of rsIL-6R. Purified protein or infected cell supernatants (24 h) were run on a polyacrylamide gel, transferred onto a PVDF membrane, analyzed by Western blot with specific purified IgG anti-rat sIL-6R and revealed with a chemiluminescent detection assay. Samples were: purified recombinant rat sIL-6R expressed in *E. coli* (lane 1) or insect cells (lane 2); recombinant adenovirus, expressing rat sIL-6R (Ad5.rsIL-6R (lanes 3 and 5), or control virus Ad5.LacZ (lanes 4 and 6)) infected cells supernatants (H35 cells (lanes 3 and 4) or HepG2 cells (lanes 5 and 6)).

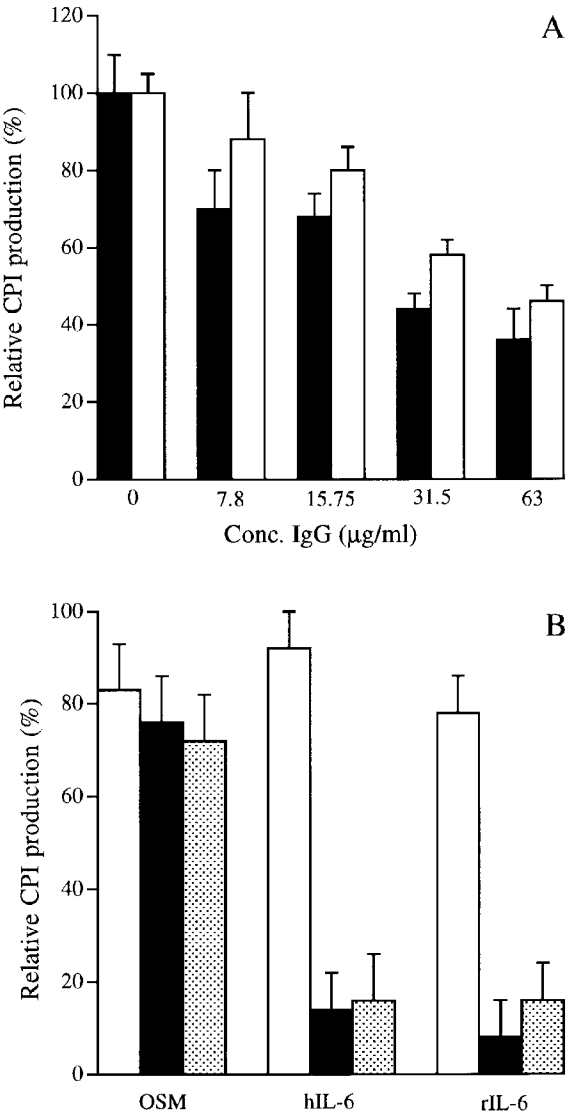


Fig. 2. Specific IgG or Fab inhibit IL-6-induced CPI synthesis from H35 cells. H35 cells were stimulated for 2 days with 2 μg/ml of rat IL-6, in the presence of increasing amounts of anti-rat sIL-6R (filled box) or anti-rat IL-6 (empty box) IgG and supernatants were analyzed for CPI content. Results are expressed as the mean of 3 different experiments with standard error; B: H35 cells were stimulated with 50 ng/ml of human OSM, 100 ng/ml of human IL-6 or 500 ng/ml of rat IL-6 in presence of control IgG (50 μg/ml, empty box), anti-rat sIL-6R specific IgG (32 μg/ml, filled box) or anti-rat sIL-6R specific Fab fragments (50 μg/ml, dotted box). CPI concentration was determined after a 2 day stimulation. Results are expressed as the mean of 3 different experiments with standard error.

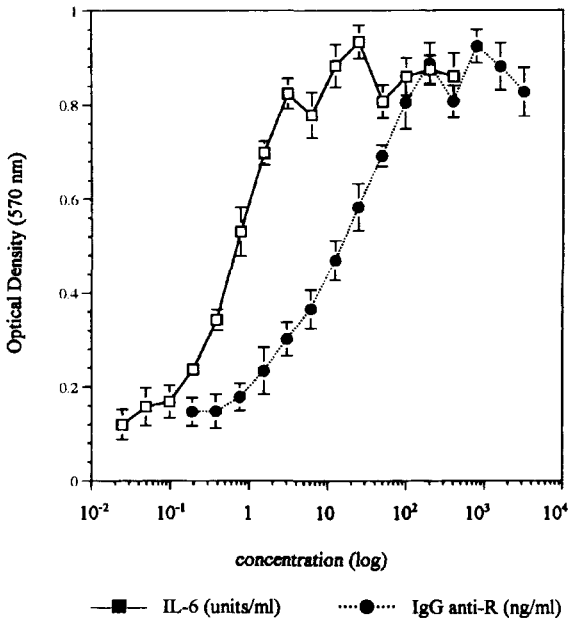


Fig. 3. rsIL-6R-specific IgG induce B9 cell proliferation. B9 cells were incubated for 72 h in presence of increasing concentrations of rat IL-6 (U/ml; open square) or rat sIL-6R (μg/ml; closed circle) specific IgG. Proliferation was measured by an MTT assay and read on a spectrophotometer at 570 nm. A representative experiment done in triplicate is shown, results are expressed as mean ± standard error.

### 3.2. *Anti-rat sIL-6R inhibits IL-6-induced acute phase response but does not modify OSM response*

To test the ability of the antibody to inhibit IL-6-induced cell responses, anti-rat sIL-6R (or purified Fab fragments or preimmune control IgG) was assessed for effects on cytokine-induced acute phase protein response of rat H35 and human HepG2 hepatoma cells. Increasing concentrations of anti-rat sIL-6R specific IgG inhibited IL-6-induced α1-cysteine protease inhibitor (CPI, a typical acute phase protein) production from H35 cells in a dose-dependent manner (Fig. 2A). At low concentration of anti-rat sIL-6R (7.8 μg/ml), we could inhibit more than 20% of CPI production induced with 2 μg/ml rat IL-6 and at 63 μg/ml, we obtained a 61% inhibition, similar to that seen with an equivalent amount of a neutralizing antibody against IL-6 ligand. 63 μg/ml anti-rat sIL-6R also inhibited 85% of CPI secretion by H35 cells that were stimulated with 100ng/ml of human IL-6. Neither control IgG nor specific anti-rat sIL-6R modified the spontaneous synthesis of acute phase proteins, and control IgG had no influence on IL-6-induced CPI production (not shown).

In order to better characterize the specificity of this effect, we analyzed CPI production in H35 cells that were stimulated with 50 ng/ml of OSM in the presence of specific or control IgG (Fig. 2B). OSM induces CPI in these cells but does not interact with the IL-6R. Use of inhibitory concentrations of specific IgG anti-rat sIL-6R (63 μg/ml) did not modify OSM-induced CPI production, suggesting the antibody was acting at the IL-6R gp80 level and not on the gp130 transducer protein. In order to rule out a non-specific effect of IgG molecules at the cell surface, we generated Fab fragments of the anti-rat sIL-6R by papain cleavage. Used at similar concentrations to intact IgG, purified Fab fragments (24 μg/ml) showed a similar inhibitory effect on rat IL-6-induced acute phase protein production (Fig. 2B). Experiments carried out on human HepG2 cells did not show inhibitory action of the anti-rat sIL-6R on human IL-6-induced responses (not shown).

### 3.3. *Anti-rat sIL-6R specific IgG can induce proliferation of B9 cells, whereas Fab fragments inhibit*

Proliferation of the B9 hybridoma cell line is widely used as a sensitive assay of IL-6 biological activity. Surprisingly, and contrary to what we observed on rat hepatoma cells, specific IgG anti-rat sIL-6R alone showed a strong agonistic effect (Fig. 3). As low as 1 ng of specific IgG could induce B9 cell growth with maximum proliferation induced with 300 ng. The dose response was roughly similar in profile to the IL-6 response curve, and had similar maximum values. By comparison, 20 ng of specific IgG could induce proliferation equal to that seen with 1 U of IL-6. In contrast to specific IGg anti-rat sIL-6R, purified Fab fragments had no stimulatory effect on proliferation. When B9 cells were incubated in presence of 2 U of IL-6, increasing concentration of Fab fragments markedly inhibited IL-6-induced proliferation (Fig. 4), similar to inhibitory effects of Fab fragments on CPI production by H35 cells (Fig. 2B). Thus monovalent specific antibody was inhibitory, but bivalent cross-linking antibody was stimulatory in B9 cells.

### 3.4. *Anti-rat sIL-6R induces STAT activation in B9 cells, but not other IL-6-responsive cells*

To further explore the mechanism by which anti-rat sIL-6R activates B9 cells, we examined the activation of STAT proteins in nuclear extracts of stimulated cells. IL-6 stimulation of B9 cells induced a strong activation of STAT-binding activity as shown by gel shift assay (Fig. 5) as has been established previously. Lane 4 (human IL-6, 400 U/ml) and 5, 6, 7

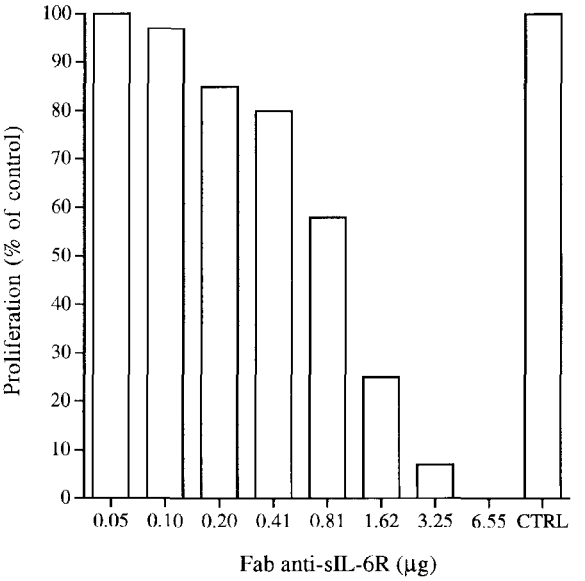


Fig. 4. rsIL-6R-specific Fab fragments prevent IL-6-induced B9 cell proliferation. B9 cells were incubated for 72 h in the presence of 2 U of rat IL-6 in 96 well plates with addition of 50 μg/ml control IgG (CTRL) or rat sIL-6R-specific Fab fragments at increasing concentrations. Results are expressed as percentage of proliferation as compared to cells incubated with similar concentrations of control IgG. A representative experiment is shown.

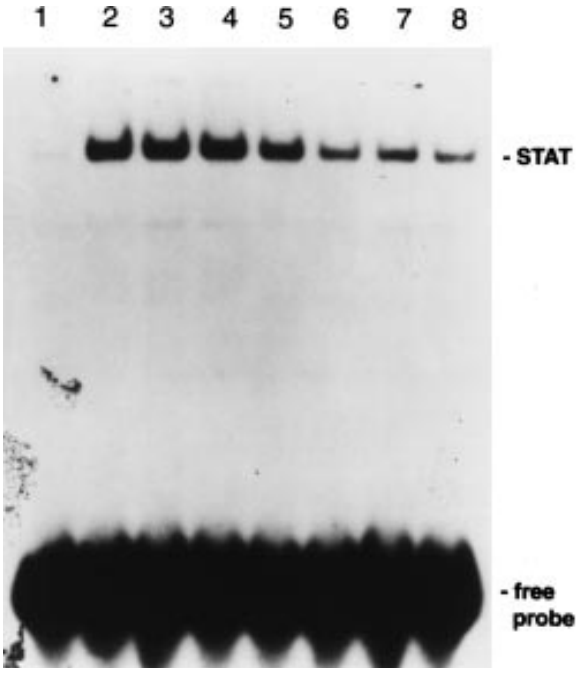


Fig. 5. STAT activation in B9 cells. Cells were untreated (lane 1), treated with IgG anti-rat sIL-6R (63 μg/ml, lane 2 or 126 μg/ml, lane 3), hIL-6 (400 U/ml, lane 4), rIL-6 (16, 1 or 0.25 U/ml, lanes 5, 6 and 7 respectively) or a combination (lane 8) of rIL-6 (16 U/ml) and Fab anti sIL-6R (30 μg/ml). Gel shift analysis was performed as in Section 2 with SIE probe and nuclear extracts. The gel shifts corresponding to STAT activated complexes are indicated.

(16, 1, 0.25 U/ml respectively of rat IL-6) show a dose-dependent induction with detection of specific bands for STAT dimers. This probe detects binding of STAT-1 and STAT-3 homo- and hetero-dimers which migrate closely together (Fig. 5) but can be seen as separate bands when run longer on gels (Fig. 6). These bands were supershifted by anti-STAT antibodies (not shown). In the presence of specific IgG anti-rat sIL-6R alone (lanes 2 and 3, 63 and 126 μg/ml of IgG respectively), STAT activation was strongly induced in B9 cell extracts, to an extent similar to that seen with high concentrations of IL-6 (Fig. 5). In contrast, stimulation of B9 cells with IL-6 in the presence of Fab fragments of anti-rat sIL-6R dramatically reduced IL-6-induced STAT activation (compare lane 5 versus 8). The same amount of Fab fragments inhibited proliferation by approximately 80%.

In H35 cells (Fig. 6) IL-6 induced high amounts of STAT binding to the SIE probe at 15 min after stimulation. Treatment with the specific antibody to sIL-6R alone did not alter levels of signal, however in combination with IL-6, the antibody dramatically inhibited STAT activation as did the Fab fragments. We also tested the action of the antibody on other IL-6-responsive mouse cell lines, including the myeloid leukemia cell line M1, and an embryonic fibroblast cell line N1H-3T3. In both cell types, IL-6-induced marked STAT activation, and inclusion of anti-rat sIL-6R IgG inhibited the IL-6 effect. The antibody alone was again without detectable effects on STAT. In contrast, shown in the top panel of Fig. 6, a parallel experiment with B9 cells shows STAT activation by IgG anti-rat sIL-6R alone, and augmentation of STAT binding with IL-6 and antibody treatment.

## 4. Discussion

We have generated antibodies against the rat soluble IL-6 receptor, which specifically recognize the receptor as demonstrated by its detection of a single band on Western blot when used to probe recombinant sIL-6R protein from various expression systems (Fig. 1). Our experiments on rat H35 hepatoma cells clearly demonstrate the ability of specific IgG directed against rat sIL-6R to block IL-6-induced acute phase protein production (Fig. 2). This was specific for IL-6R-mediated events since OSM stimulation of cells was not affected. OSM has been recently shown to act through at least two different receptor complexes, the LIF receptor (LIFR) and the specific Oncostatin-M receptor (OSMR) [25,26], both of which require gp130 for signalling. The antibody did not affect HepG2 cells, consistent with low homology between rat and human receptors [27]. Non-specific effects due to Fc portion of IgG molecules were ruled out on the basis of Fab fragment experiments that confirmed reactivity with specific epitomes of gp80 (Fig. 2).

As it is now well established, IL-6 by interacting with its specific receptor induces B9 cell proliferation. Receptor-ligand interaction induces STAT activation, the role of which in proliferation is not yet fully delineated. Alternatively, other pathways such as MAP kinases, triggered after Jak phosphorylation [28], may be involved in B9 cell proliferation. Contrary to expectations, our results showed that specific IgG antibodies directed against gp80-induced STAT activation and a dramatic proliferation similar to that induced by IL-6 (Fig. 3), whereas Fab fragments were inhibitory (Figs. 3–6). Recent studies by others have shown that cross-linking of human gp130 with a specific antibody can induce Jak kinase and

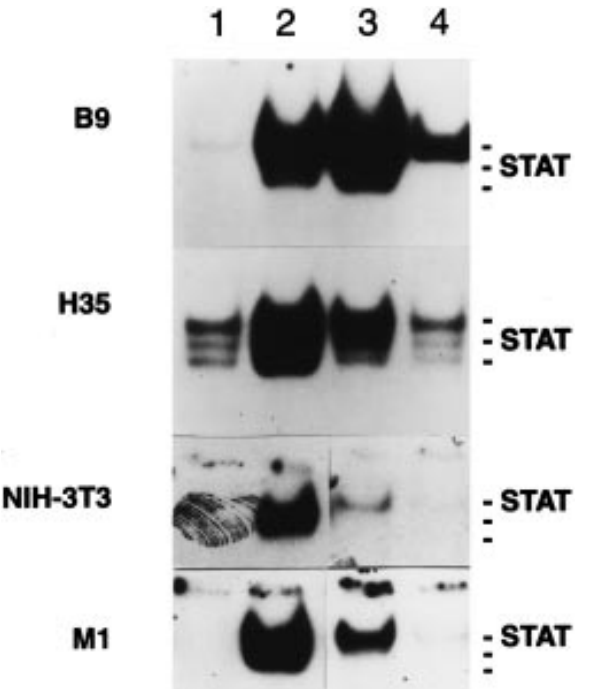


Fig. 6. comparison of STAT activation in B9, H35, 3T3-NIH and M1 cells. Cells were untreated (lane 1), treated with rIL-6, 16 U/ml (lane 2) anti-rat sIL-6R, 63 μg/ml (lane 3) or the combination (lane 4) as indicated. Gel shift analysis was performed as in Section 2 with SIE probe and nuclear extracts. The gel shifts corresponding to STAT activated complexes are indicated.

biologic responses in HepG2 cells and hematopoietic cells [29]. We suggest that in B9 cells treated with specific IgG anti-rat sIL-6R, two molecules of gp80 are cross-linked sufficiently to induce activation of Jak kinase(s) and subsequent STAT activation and a proliferation signal. Fab fragments, on the other hand, exhibit monovalent reactivity with antigen, cannot induce dimerization and therefore block the ligand site and any IL-6-induced effects.

Interestingly, this apparently does not occur in other IL-6-responsive cells including rat H35 hepatoma cells, mouse NIH-3T3 fibroblasts and mouse M1 cells, where IgG anti-rat sIL-6R inhibited signalling events, as measured by STAT activation (Fig. 6). Since IgG anti-rat sIL-6R reacts specifically with gp80 and not with gp130, our experiments imply either that gp80, at least on B9 cells, are pre-associated with gp130 free of IL-6 or with a residual amount of ligand already bound to it. We and others have previously noted the possibility that B9 cells can secrete low amounts of IL-6 [12,16,19]. This could explain the proliferation activity of recombinant sIL-6R by itself without addition of exogenous IL-6. This sIL-6R agonist activity has not been reported for other cell lines and it is therefore very likely that the B9 cell IL-6R/gp130 complex differs slightly from that described for other cell lines. Alternatively, binding of specific antibody to gp80 may induce a conformational change allowing for the binding of gp80 with gp130. Others have reported agonistic activities of receptor domain (WSEWS) specific monoclonal antibodies on HepG2 cells [30], also suggesting cross-linking of receptor components can induce signals. In addition, activation of several Jak kinases in the IL-6 signalling pathway have already been demonstrated by several authors [7,31–33]. Thus the difference between B9 cells and the other cells tested could be due to relative amounts of separate Jak kinase family members which differ in response to cross-linking of gp80. Further experimentation is needed to determine the exact nature of these differences in IL-6 signalling.

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## References

[1] T. Kishimoto, S. Akira, M. Narazaki, T. Taga, Blood 86 (1995) 1243–1254.
[2] D. Pennica, K.J. Shaw, T.A. Swanson, M.W. Moore, D.L. Shelton, K.A. Zioncheck, A. Rosenthal, T. Taga, N.F. Paoni, W.I. Wood, J. Biol. Chem. 270 (1995) 10915–10922.
[3] L.D. Ward, G.J. Howlett, G. Discolo, K. Yasukawa, A. Hammacher, R.L. Moritz, R.J. Simpson, J. Biol. Chem. 269 (1994) 23286–23289.
[4] G. Paonessa, R. Graziani, A. De Serio, R. Savino, L. Ciapponi, A. Lahm, A.L. Salvati, C. Toniatti, G. Ciliberto, EMBO J. 14 (1995) 1942–1951.
[5] J.N. Ihle, I.M. Kerr, Trends Genet. 11 (1995) 69–74.
[6] J.N. Ihle, Adv. Immunol. 60 (1995) 1–35.
[7] C. Lutticken, U.M. Wegenka, J. Yuan, J. Buschmann, C. Schindler, A. Ziemiecki, A.G. Harpur, A.F. Wilks, K. Yasukawa, T. Taga, et al. Science 263 (1994) 89–92.
[8] N. Stahl, T.J. Farruggella, T.G. Boulton, Z. Zhong, J.E. Darnell, G.D. Yancopoulos, Science 267 (1995) 1349–1353.
[9] T. Kishimoto, Stem Cells 12, Suppl. 1 (1994) 37–44; discussion 44–45.
[10] C. Lutticken, P. Coffer, J.P. Yuan, C. Schwartz, E. Caldenhoven, C. Schindler, W. Kruijer, P.C. Heinrich, F. Horn, FEBS Lett. 360 (1995) 137–143.
[11] A. Mackiewicz, H. Schooltink, P.C. Heinrich, S. Rose-John, J. Immunol. 149 (1992) 2021–2027.
[12] C. Gabay, P. Silacci, B. Genin, G. Mentha, C. Le Coultre, P.A. Guerne, Eur. J. Immunol. 25 (1995) 2378–2383.
[13] K. Yoshida, I. Chambers, J. Nichols, A. Smith, M. Saito, K. Yasukawa, M. Shoyab, T. Taga, T. Kishimoto, Mech. Dev. 45 (1994) 163–171.
[14] T. Tamura, N. Udagawa, N. Takahashi, C. Miyaura, S. Tanaka, Y. Yamada, Y. Koishihara, Y. Ohsugi, K. Kumaki, T. Taga, et al. Proc. Nat. Acad. Sci. USA 90 (1990) 11924–11928.
[15] T. Taga, M. Hibi, Y. Hirata, K. Yamasaki, K. Yasukawa, T. Matsuda, T. Hirano, T. Kishimoto, Cell 58 (1989) 573–581.
[16] M. Diamant, M.B. Hansen, K. Rieneck, M. Svenson, K. Yasukawa, K. Bendtzen, J. Immunol. Methods 173 (1994) 229–235.
[17] D.P. Gearing, C.J. Thut, T. VandeBos, S.D. Gimpel, P.B. Delaney, J. King, V. Price, D. Cosman, M.P. Beckmann, EMBO J. 10 (1991) 2839–2848.
[18] S. Rose-John, P.C. Heinrich, Biochem. J. 300 (1994) 281–290.
[19] V. Thibault, B. Terlain, J. Gauldie, J. Interferon Cytokine Res. (1996) in press.
[20] T. Mosman, J. Immunol. Methods 65 (1983) 55–60.
[21] L. Aarden, A. De Groot, O.L. Schaap, P.M. Lansdorp, Eur. J. Immunol. 17 (1987) 1411–1416.
[22] T.A. Braciak, W. Northemann, D.K. Chong, J.A. Schroeder, J. Gauldie, Prot. Express. Purif. 7 (1996) 269–274.
[23] C.D. Richards, C. Langdon, F. Botelho, T.J. Brown, A. Agro, J. Immunol. 156 (1996) 343–349.
[24] H.B. Sadowski, K. Shuai, J.E. Darnell Jr., M.Z. Gilman, Science 261 (1993) 1739–1744.
[25] D.P. Gearing, M.R. Comeau, D.J. Friend, S.D. Gimpel, C.J. Thut, J. McGourty, K.K. Brasher, J.A. King, S. Gillis, B. Mosley, et al. Science 255 (1992) 1434–1437.
[26] B. Mosley, C. Delmus, D. Friend, N. Boiani, B. Thoma, L.S. Park, D. Cosman, J. Biol. Chem. 271 (1996) 32635–32643.
[27] M. Baumann, H. Baumann, G.H. Fey, J. Biol. Chem. 265 (1990) 19853–19862.
[28] G. Kumar, S. Gupta, S. Wang, A.E. Nel, J. Immunol. 153 (1994) 4436–4447.
[29] M. Fourcin, S. Chevalier, C. Guillet, O. Robledo, J. Froger, A. Pouplardbarthelaix, H. Gascan, J. Biol. Chem. 271 (1996) 11756–11760.
[30] J. Biro, S. Bosze, F. Hudecz, Z. Nagy, E. Rajnavolgyi, B. Schmidt, E. Rakasz, A. Falus, Immunol. Lett. 46 (1995) 183–187.
[31] L.C. Berger, T.S. Hawley, J.A. Lust, S.J. Goldman, R.G. Hawley, Biochem. Biophys. Res. Commun. 202 (1994) 596–605.
[32] T. Matsuda, T. Hirano, Blood 83 (1994) 3457–3461.
[33] N. Stahl, T.G. Boulton, T. Farruggella, N.Y. Ip, S. Davis, B.A. Witthuhn, F.W. Quelle, O. Silvennoinen, G. Barbieri, S. Pellegrini, et al. Science 263 (1994) 92–95.