In Vivo Biological Effects of Recombinant Soluble Interleukin-4 Receptor (43750)

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Abstract. **We investigated the role of soluble interleukin-4 receptor (sIL-4R) as a regulator of IL-4 mediated activities *in vivo*. Administration of recombinant sIL-4R to mice resulted in (i) prolonged survival of heterotopic cardiac allografts; (ii) decreased popliteal lymph node enlargement in response to allogeneic cells; and (iii) inhibition of IgE secretion in response to anti-IgD treatment. Transgenic mice constitutively expressing elevated levels of biologically active sIL-4R displayed prolonged cardiac allograft survival compared with control animals. However the sIL-4R transgenic mice were capable of mounting normal antigen-specific IgE responses despite the presence in serum of up to 3 μg/ml sIL-4R. Surprisingly, coadministration of IL-4/sIL-4R or IL-4/anti-IL-4 mAb complexes caused a superinduction of IgE secretion in anti-IgD-treated normal mice and subsequently in other IL-4-dependent biological activities. Thus, recombinant sIL-4R can not only antagonize functions mediated by endogenous IL-4, but also potentiate the biological activity of exogenously administered IL-4. These dual roles may have possible clinical implications for the recombinant molecule, as well as for natural sIL-4R immunoregulation.** [P.S.E.B.M. 1994, Vol 206]

Interleukin-4 (IL-4) acts at numerous junctures in the immune response (reviewed in 1 and 2). As "B cell stimulatory factor-1" (BSF-1), IL-4 induces B cell activation, growth, and differentiation into immunoglobulin secreting cells *in vitro*. Studies with normal and IL-4 deficient mice have confirmed that IL-4 is required for the generation of IgE responses *in vivo* (3, 4). The range of IL-4 biological functions extends to other cell types including T cells, macrophages, mast cells, and hematopoietic progenitor cells, and all of these activities are mediated by a distinct cell receptor. The IL-4 receptor is a member of the hematopoietin/growth factor receptor gene superfamily, as defined by the presence of highly conserved cysteine residues and the WSXWS motif, each of which is contained within less well conserved extracellular domains (5).

During the cloning of membrane-bound IL-4R, Mosley *et al.* identified an mRNA transcript encoding a putative soluble IL-4 receptor (sIL-4R) which lacks the transmembrane and cytoplasmic regions of the 140-kDa full-length receptor (6). The recombinant sIL-4R has been expressed in transfected mammalian cells as a differentially glycosylated 37- to 40-kDa protein (7) and corresponds structurally and immunologically to a natural IL-4-binding protein found in biological fluids of mice (8, 9). Chilton and Fernandez-Botran recently showed that the natural sIL-4R is produced by T cells, B cells, and macrophages and that its synthesis is stimulated by IL-4 itself (10). The latter finding suggests a mechanism by which IL-4 can regulate its own activity.

One possible biological role of natural sIL-4R is its competition with cell surface IL-4R for IL-4 binding, thereby acting as an IL-4 antagonist. Alternatively sIL-4R might serve as an IL-4 transporter by enhancing its availability to cell surface receptors and, consequently, its overall activity. Each of these possibilities would have important implications not only for

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normal immunoregulation but also for the potential use of sIL-4R in clinical settings.

Functional and Pharmacokinetic Characterization of Recombinant sIL-4R

The murine recombinant sIL-4R purified from transfected HeLa cells retained the ability to bind to IL-4. It specifically blocked IL-4 binding to B cells, with a $K_i = 4.8 \times 10^9\, M^{-1}$, which is equivalent to that of the 11B11 anti-IL-4 mAb used in several of the studies described in this review (11). The sIL-4R was also able to inhibit the following IL-4-mediated activities *in vitro:* (i) proliferation of the CTLL-2 T cell line (6); (ii) enhanced B cell surface expression of Class II MHC and low-affinity IgE Fc receptors (11); (iii) proliferation of B cells in the presence of the costimulus anti-immunoglobulin (11); and (iv) induction of IgE and IgG1 class switching and secretion in the presence of lipopolysaccharide (11). These *in vitro* studies illustrated that recombinant sIL-4R is a potent and specific antagonist of IL-4 binding and bioactivity.

The pharmacokinetic parameters of recombinant sIL-4R were determined by administering the radiolabeled cytokine to mice (7). Intravenously injected sIL-4R had a distribution half-life of 9 min, with liver and kidney as the primary and secondary sites of distribution. The elimination half-life was 2.3 hr, with complete elimination by 11 hr occurring primarily through the liver and kidneys. The highest cumulative tissue distribution occurred in the blood. Intraperitoneal or subcutaneous administration resulted in prolonged elimination half-lives of 4.2 and 6.2 hr, respectively. Although the half-life of sIL-4R does not approach those reported for antibodies, it is considerably more persistent than cytokines in general. Moreover, the determination of sIL-4R biodistribution and elimination parameters established guidelines by which to construct protocols for sIL-4R bioactivity studies *in vivo*.

Recombinant sIL-4R as Antagonist of IL-4 Activities *In Vivo*

The *in vivo* biological activity of recombinant sIL-4R was first tested in two different murine models for alloreactivity. In the lymph node hyperplasia host versus graft model (12), BALB/c mice were injected with irradiated allogeneic C57Bl/6 splenocytes in one footpad and with irradiated syngeneic BALB/c splenocytes in the contralateral footpad. Seven days later, draining popliteal lymph nodes were removed and weighed. As expected, enlargement of the popliteal lymph node (PLN) draining the site of the allogeneic cell injection was observed compared with the contralateral PLN. However, considerably less hyperplasia was observed in mice that had been treated intraperitoneally with sIL-4R on Day -1, 0, and $+1$ relative to the time of allogeneic cell injection. This effect was sIL-4R dose-dependent, with as little as 100 ng/day causing greater than 50% inhibition of the response. Coadministration of IL-4 with sIL-4R reversed this effect, which indicated that the effect was cytokine specific. sIL-4R appeared to act early in the response, since delay of administration until Day 1 post challenge resulted in no inhibition of lymph node enlargement. The 11B11 IL-4 neutralizing mAb also blocked the PLN response, but considerably higher levels were required to achieve the same level of inhibition observed with the sIL-4R.

In the heterotopic heart allograft model (13) mice were engrafted with allogeneic newborn hearts in the ear pinnae and transplant viability was measured daily by visual observation of pulsatile activity in the ear. The transplants lasted an average of about 11 days in control mice treated with mouse serum albumin on Day 0, 1, and 2, relative to time of transplant. Administration of sIL-4R (1 μg/day) over the same time period prolonged survival time to a mean of about 15 days, which was determined to be statistically significant. The effect was dose-dependent because sIL-4R administered at 100 ng/day had no effect upon allograft survival. The results from these allograft models illustrated two important points. First, they confirmed the hypothesis that IL-4 is a critical component of *in vivo* allogeneic responses. Second, they showed for the first time that recombinant sIL-4R can act as an inhibitor of *in vivo* IL-4 activities. More recent findings demonstrate that *in vitro*-activated Th2 cells can mediate local tissue inflammation *in vivo,* a response which can be blocked by early administration of sIL-4R or anti-IL-4 mAb (12).

Studies using several *in vivo* models have demonstrated that IL-4 is absolutely required for the generation of IgE responses (reviewed in 3). For example, treatment of mice with anti-IgD stimulates a large increase in IgE production, and this effect can be completely blocked by administration of an anti-IL-4 mAb. Anti-IL-4 mAb also blocks increases in serum IgE accompanying acute and chronic nematode infections, and suppresses the development of primary and secondary antigen-specific IgE responses. We used the anti-IgD immunization model to assess the ability of recombinant sIL-4R to antagonize IgE production (14). Mice were immunized with anti-IgD, followed by twice-daily intraperitoneal injections of sIL-4R or anti-IL-4 mAb on Day 3, 4, and 5. Immunoglobulin levels in Day 9 sera from these animals were determined by ELISA. Serum from anti-IgD-treated Balb/c mice contained approximately 20 μg/ml IgE compared with 300 ng/ml in control mice. Administration of 30–1000 μg/day of recombinant murine sIL-4R caused a dose-dependent reduction in anti-IgD induced IgE secretion, with 85% inhibition achieved with the highest

dose of sIL-4R. Recombinant human sIL-4R had no effect, consistent with the species specificity of IL-4 receptor binding. The murine sIL-4R was not nearly as effective an inhibitor as a neutralizing anti-IL-4 mAb. For instance, administration of as little as 30 μg/day of anti-IL-4 mAb reduced the amount of IgE produced from approximately 19 μg in control animals to 2 μg in treated animals, whereas 1 mg of sIL-4R was required for a similar inhibitory effect. In the host versus graft alloreactivity model sIL-4R was a more potent inhibitor than anti-IL-4 mAb.

We then created transgenic mice in which sIL-4R was constitutively expressed under control of the metallothionein promoter (15). ELISA and IL-4 binding assays demonstrated that serum levels of biologically active sIL-4R in transgenic animals (810–2700 ng/ml) greatly exceeded those in littermate controls (19–33 ng/ml). Phenotypic characterization of lymphoid organs in sIL-4R transgenic mice revealed normal numbers of B and T cells and normal surface marker expression. Splenic lymphocytes displayed normal antigen-specific antibody responses and generation of cytotoxic T cells *in vitro*.

Despite the normal phenotypes displayed by the sIL-4R transgenic mice, we expected that these animals would be deficient in allogeneic responsiveness due to the high circulating sIL-4R levels. To test this hypothesis, both transgenic and littermate control mice were given heart allografts, and survival times were determined. When heterotopic hearts were transplanted into the ears of littermate control mice the allografts survived an average of 9.5 days. By contrast, cardiac allograft survival in the sIL-4R transgenic mice was prolonged by 45% compared with nontransgenic littermate controls, thus supporting our previous finding that microgram quantities of sIL-4R are sufficient to block allogeneic responses (12).

We further hypothesized from the high serum sIL-4R levels that the transgenic mice would also be deficient in their ability to mount an antigen-specific IgE response. Transgenic and control mice were primed with the antigen TNP-KLH and reimmunized 21 days later. Five days after boosting, serum samples were tested for TNP-specific IgE levels. This protocol typically induces high levels of both polyclonal and TNP-specific IgE and IgG1 in normal mice, and the effect on IgE production can be blocked by anti-IL-4 mAb (16). We were therefore surprised to find that both littermate control and sIL-4R mice displayed strong antigen-specific IgE responses, and there was no correlation between anti-TNP IgE and sIL-4R levels in serum from individual transgenic mice (15). Thus, the results demonstrated that constant circulating levels of sIL-4R in excess of 3 μg/ml are not sufficient to block IgE production, although they do account for the delayed allograft rejection seen in transgenic mice.

The findings from normal mouse models and sIL-4R transgenic mice indicated that considerably higher levels of sIL-4R are required to block IgE secretion relative to allogeneic responsiveness. In the case of IgE responses, this requirement may be explained in part by considering that cognate interaction between IL-4-secreting T cells and IL-4-responsive B cells is intimate (17). In such a situation, very high local concentrations of IL-4 would be available for uptake by B cell surface IL-4R; thus, high local concentrations of sIL-4R would be required to antagonize IgE production. By contrast, IL-4 generated in the allogeneic response models may be acting distal to its site of secretion. Thus, locally produced IL-4 may diffuse or circulate to sites of inflammation, where one reported role for IL-4 is the enhancement of VCAM-1 expression on endothelial cells (18, 19) which is followed by increased leukocyte adhesiveness and their subsequent transendothelial migration. In such a scenario, the effect of IL-4 may be more easily antagonized by sIL-4R because of accessibility and relatively low levels of IL-4 available to membrane IL-4R on endothelial cells or other relevant targets.

Potentiation of IL-4 Activities *In Vivo*

An interesting phenomenon occurred when we attempted to determine whether exogenous IL-4 could reverse the inhibitory effects of sIL-4R in the anti-IgD induced IgE secretion model. Anti-IgD immunized mice were treated on Day 3 through 5 with sIL-4R, IL-4, or complexes of IL-4/sIL-4R or IL-4/anti-IL-4, then serum IgE levels were measured on Day 9 (14). As expected, sIL-4R alone blocked IgE secretion, whereas the administration of IL-4 alone to anti-IgD mice failed to appreciably increase already robust IgE levels. Unexpectedly, IL-4/sIL-4R complexes caused a superinduction (3- to 6-fold increase) of IgE secretion over levels induced by anti-IgD by alone. The superinductive effect of IL-4/sIL-4R complexes was dependent upon the relative amounts of soluble receptor and ligand administered. That is, coadministration of IL-4 with midrange concentrations of sIL-4R was stimulatory, but a potentiating effect was not observed at lower concentrations of sIL-4R. High concentrations of sIL-4R reversed the potentiating effect. We observed similar effects when complexes of anti-IL-4 mAb and IL-4 were administered to anti-IgD-stimulated mice, although the dose-response curves differed from those obtained with sIL-4R/IL-4 complexes. In fact, on a molar basis, the anti-IL-4 mAb was a more effective enhancer of exogenous IL-4 activity.

The concept of cytokine agonist activity was further explored by administering IL-4/anti-IL-4 complexes to mice and measuring cell surface Ia expression on splenocytes (20). A single injection of IL-4/

anti-IL-4 complexes induced a 5-fold increase in Ia expression, and levels remained elevated for 5 days. Mice treated with IL-4 alone displayed a relatively small, short-lived increase in Ia expression. It was also demonstrated that (i) increased Ia expression was dependent upon the ratio of IL-4 to anti-IL-4 mAb; (ii) complexes formed with non-neutralizing anti-IL-4 mAb had no effect, and (iii) the increase in Ia expression was blocked by anti-IL-4R mAb. Similar effects have been observed in other models as well. For instance, Urban *et al.* (21) demonstrated that IL-4/anti-IL-4 complexes decreased worm survival in T cell-depleted mice infected with the nematode parasite *Nippostrongylus brasiliensis*. In another study, Else *et al.* (22) demonstrated that IL-4/anti-IL-4 complexes protected otherwise susceptible mice from chronic infection with the nematode parasite *Trichuris muris*. Thus, complexes of IL-4 and IL-4-binding proteins can generate responses *in vivo* that significantly exceed those induced by IL-4 alone.

How do soluble cytokine receptors or anti-cytokine mAbs enhance the *in vivo* activities of exogenously administered cytokines? Fernandez-Botran and Vitetta (23) addressed this issue in studies involving the natural sIL-4R. They demonstrated that (i) IL-4 dissociated more rapidly from sIL-4R than from cell surface IL-4R at 37°C; (ii) following dissociation from sIL-4R, IL-4 could bind to the cell surface receptor; and (iii) sIL-4R protected IL-4 from proteolytic degradation. Thus, the natural sIL-4R can serve as a carrier protein for IL-4, perhaps by enhancing the effective half-life of IL-4 and delivering the cytokine to cells bearing IL-4 receptors.

Conclusions

We are faced with the possibility that under different circumstances sIL-4R, and perhaps soluble cytokine receptors in general, may act as either antagonists or potentiators of cytokine activities. The pharmacokinetics of IL-4/sIL-4R complexes and of their individual components, the relative concentrations and distribution of IL-4 and sIL-4R, the nature of the IL-4-mediated response (local versus systemic; relative apposition of cells secreting and responding to IL-4), and other phenomena could all help to determine the role that sIL-4R plays in a particular setting. The relevance of the aforementioned findings to normal immunoregulatory processes is as yet unclear; we still do not know whether natural sIL-4R can serve a dual role in enhancing or blocking endogenous IL-4. Nonetheless, these observations do point to potential clinical applications for sIL-4R under either guise. As an antagonist, sIL-4R may be effective in reversing Th2 dominated responses and eliciting Th1-like responses. An obvious application is in allergy/asthma, where IL-4 could play a critical role through its purported ability to induce a shift toward Th2 cell development (24, 25), stimulate IgE secretion (3), enhance expression of low-affinity IgE Fc receptors (26), and induce mastocytosis (27) and eosinophilia (28). Alternatively, IL-4/sIL-4R complexes might be useful in clinical settings in which Th2-like responses direct more favorable outcomes as, for example, in helminthic infections (29). The next few years will be an exciting time as research emphasis shifts from preclinical studies toward therapy with sIL-4R and other soluble receptors.

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