tected and cleaved in two steps from the resin with anhydrous fluorhydric acid. After purification by exclusion chromatography and high-performance liquid chromatography, the purity and identity of each peptide were checked by amino acid analysis and microsequencing.

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14. Seven groups of female rabbits (Grand Fauve de Bourgogne, Centre d'élevage Gontrand Achard de la vente, Orne, France), consisting of two to ten animals each, received primary immunizations of peptide-carrier conjugate emulsified in complete Freund's adjuvant at multiple intradermal sites. All rabbits received 200 μg of equivalent peptide except those injected with a peptide from batch 7, which received only 15 μg of equivalent peptide. Secondary immunizations were performed with the same quantities of immunogen in incomplete Freund's adjuvant injected subcutaneously 10, 20, 30, and 40 days after the first immunization. Sera were collected before immunizations and on days 23, 33, and 43 after the first injection and stored at −20°C until assayed.
15. Peptide-coated microtiter plates were incubated with various dilutions of antisera [P. Motte, G. Alberici, M. Ait-Abdellah, D. Bellet, *J. Immunol.* **138**, 3332 (1987)]. After washing, the plates were incubated with a 1:1000 dilution of peroxidase-conjugated rabbit antibodies to mouse immunoglobulin for 1 hour at 37°C. Finally, enzyme activity was determined with *o*-phenylenediamine as a chromogenic substrate, and absorbance was measured at 492 nm.
16. The iodogen method was used to label hCG, hCGα, and hCGβ (100 μCi/μg; 1 Ci = 3.7×10^{10} Becquerels) [P. J. Fraker and J. C. Speck, *Biochem. Biophys. Res. Commun.* **80**, 849 (1978)]. ^{125}I-labeled hLH was purchased from the Commissariat à l'Energie Atomique, Saclay, France. Radiolabeled hormones or subunits (30,000 cpm) were incubated overnight at 4°C with serial dilutions of antiserum. The antigen-antibody complex was precipitated by polyethylene glycol and, after centrifugation, the radioactivity of the pellet was counted [J. M. Bidart *et al.*, *J. Biol. Chem.* **262**, 8551 (1987)].
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19. Serial dilutions of recombinant products (50 μl) were incubated with the dilution of antiserum (100 μl) which produced 50% binding to ^{125}I-hCG, and with ^{125}I-hCG (30,000 cpm, 50 μl). After an overnight incubation at 4°C, the ^{125}I-hCG–antibody complex was precipitated by polyethylene glycol. After centrifugation, the radioactivity of the pellet was counted.
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24. Single-letter abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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Regulation of Alloreactivity in Vivo by a Soluble Form of the Interleukin-1 Receptor

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In vitro studies have shown that cytokines are involved in the regulation of the immune response, but their role in vivo is less well defined. Specific cytokine antagonists enable the identification of particular cytokines involved in the response and offer a means for modifying it. Systemic administration of a soluble, extracellular portion of the receptor for interleukin-1 (sIL-1R) had profound inhibitory effects on the development of in vivo alloreactivity. Survival of heterotopic heart allografts was prolonged from 12 days in controls to 17 days in mice treated with sIL-1R. Lymph node hyperplasia in response to a localized injection of allogeneic cells was completely blocked by sIL-1R treatment. The inhibition was overcome by simultaneous administration of interleukin-1 (IL-1); thus, sIL-1R acts by neutralizing IL-1. These results implicate IL-1 as a regulator of allograft rejection and demonstrate the in vivo biological efficacy of a soluble cytokine receptor.

INTERLEUKIN-1α AND INTERLEUKIN-1β (IL-1α and IL-1β) are cytokines involved in regulation of the immune and the inflammatory response (*1, 2*). Despite their distinct primary amino acid sequence (*3*), both IL-1α and IL-1β bind to the same specific plasma membrane receptor (*4–8*). The IL-1 receptor (IL-1R) on murine T cells was identified by cDNA expression cloning and NH_2-terminal sequence analysis as an integral membrane glycoprotein of M_r 80,000 (*9, 10*). When a cDNA encoding 316 residues of the extracellular region of the murine IL-1R is expressed in HeLa cells, they secrete a soluble IL-1 binding protein into the culture medium (*11*). Although it is not sufficient for signal transduction (*12*), this soluble, truncated receptor (sIL-1R) exhibits IL-1 binding properties similar to those of the full-length receptor (*11*) and, as such, represents a molecule with the potential to inhibit the biological activities of IL-1. To determine if sIL-1R would modulate the immune response in vivo, we measured its effects on the rejection of cardiac allografts and on the in vivo lymphoproliferation that occurs after challenge with allogeneic cells.

Neonatal C57BL/6 (H-2^b major histocompatibility complex haplotype) hearts were transplanted into the ear pinnae of BALB/c (H-2^d) hosts. Survival of the transplanted hearts was assessed by visual inspection of the grafts for pulsatile activity. Such grafts survived 11 to 13 days in individual control mice treated with mouse serum albumin (MSA) (Table 1). When allograft recipients were given three to six daily injections of sIL-1R, graft survival was prolonged in every case. The mean graft survival time in sIL-1R–treated mice (17 to 18 days) was 5 to 6 days longer than the survival time of identical grafts in control mice (Table 1). The prolongation of cardiac allograft survival achieved with this short-course treatment of sIL-1R is similar to or greater than that observed in a similar grafting system with other immunosuppressive regimens, such as treatment with antibodies to CD4 glycoprotein or total lymphoid irradiation (*13*). Prolongation of graft survival with sIL-1R was not as great as that observed after treatment with antibody to the IL-2 receptor in a different grafting model (*14, 15*). A direct comparison of these various immunosuppressive agents would be necessary to determine their relative efficacies.

The effect of sIL-1R on alloreactivity in vivo was also examined in a second experimental system, involving the localized, T cell–dependent, lymphoproliferative response to allogeneic cells. BALB/c mice were exposed to irradiated, allogeneic

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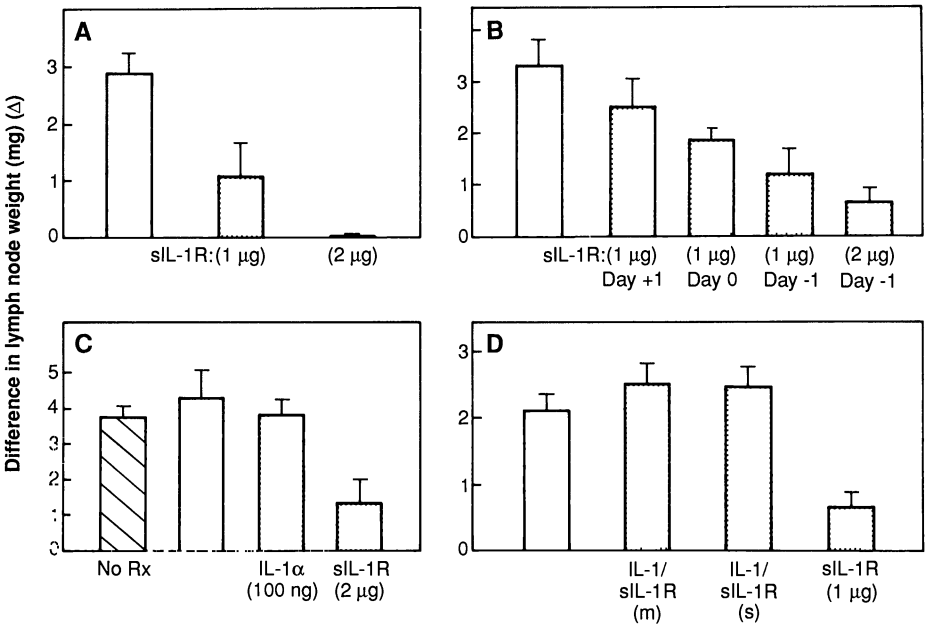

Fig. 1. Effects of sIL-1R administration on PLN response to allogeneic cells in vivo. (**A**) Inhibition of PLN enlargement by different doses of sIL-1R; (**B**) time dependence of sIL-1R treatment, with administration beginning on day −1, 0, or +1 relative to cell injection and continuing for three additional days; (**C**) effect of IL-1α or sIL-1R on PLN response, no Rx denotes no protein injection; and (**D**) administration of IL-1α reverses the inhibitory effects of sIL-1R. In (D), IL-1 and sIL-1R were either mixed before injection [IL-1/sIL-1R (m)] or injected separately [IL-1/sIL-1R (s)]. Empty bars represent control mice injected with MSA alone. The response to allogeneic cells in vivo was quantified by the PLN enlargement assay (*22*), a measure of allograft transplant immunity (*16*). The left footpads of BALB/c mice were injected intracutaneously with 10^7 irradiated, allogeneic, C57BL/6 spleen cells, and the contralateral footpads of the same mice were injected with 10^7 irradiated, syngeneic, BALB/c spleen cells. The method of sIL-1R administration and the materials used are described in (*23*). Seven days after antigen administration, the PLNs were removed and weighed, and the results are expressed as the difference in weight (in milligrams) between the lymph node draining the site of allogeneic cell injection and the node draining the syngeneic cell injection site (Δ, mean ± SEM; $n = 3$ per test group). Lymph nodes draining the syngeneic cell injection site weighed ~1.5 mg, regardless of whether they were obtained from mice treated with MSA or sIL-1R, and did not differ significantly in weight from nodes obtained from mice given no cell injection. Values for statistical significance were calculated by the two-tailed Student's *t* test. Probabilities were derived from the T distribution by means of SAS 5.18 [SAS (Statistical Analysis Systems) Institute, Cary, NC]. Where multiple comparisons were done, probabilities for significance at $P < 0.05$ were adjusted with a Bonferroni correction. (A) sIL-1R (1 μg) versus MSA, $P = 0.065$; sIL-1R (2 μg) versus MSA, $P = 0.002$*. (B) sIL-1R (1 μg beginning day −1) versus MSA, $P = 0.038$*. (C) sIL-1R (2 μg) versus MSA, $P = 0.039$*. (D) sIL-1R (1 μg) versus MSA, $P = 0.023$*; IL-1/IL-1R (s) versus IL-1R (1 μg) $P = 0.002$*. Group Δ's that are significantly different from one another at the $P < 0.05$ level with the Bonferroni correction in place are denoted by (*).

Table 1. Effects of sIL-1R treatment on nonvascularized heterotopic cardiac allograft survival. Neonatal C57BL/6 hearts were transplanted into the ear pinnae of adult BALB/c recipients as described (*19*) with slight modifications (*13, 20*). Pulsatile activity of the grafts was determined visually by examining the ear-heart grafts of anesthetized recipients under a dissecting microscope with soft reflected light beginning on day 5 or 6 after transplant. The time of graft rejection was defined as the day after transplantation on which contractile activity ceased. Recipient mice were transplanted on day 0 and injected with either sIL-1R (2 μg/day) plus MSA (100 ng) or with MSA alone on days 0 through 6, alternating intraperitoneal and subcutaneous routes (experiment 1), or on days 0 through 2, by the intraperitoneal route only (experiment 2).

Treatment group	Graft survival time (days)	Graft survival time (mean ± SD)
Experiment 1		
MSA	12, 12, 12, 13, 13, 13	12.5 ± 0.55
sIL-1R	14, 16, 17, 17, 17, 20, 21	17.4 ± 2.4*
Experiment 2		
MSA	11, 13, 13, 13	12.5 ± 1.0
sIL-1R	17, 17, 19, 20	18.3 ± 1.5*

*The probability that the graft survival time for the group treated with sIL-1R differs by chance alone from the group treated with MSA is <0.03 when analyzed by Wilcoxon rank sum test and <0.001 when analyzed by two-tailed Student's *t* test for experiment 1. The corresponding values for experiment 2 are $P < 0.06$ and $P < 0.003$.

C57BL/6 spleen cells in one footpad and irradiated, syngeneic BALB/C spleen cells in the contralateral footpad. In this system, there is an enlargement of the popliteal lymph node (PLN) draining the site of allogeneic cell injection relative to the node draining the syngeneic cell injection site, and the reaction is measured at day 7 as a gain in lymph node weight (Fig. 1) or cellularity (Table 2). Systemic administration of sIL-1R for 3 to 4 days beginning on day −1 relative to challenge with alloantigen resulted in a dramatic inhibition of this lymphoproliferative response. The effect was dose-dependent and, in some cases, the response was completely eliminated (Fig. 1A). Inhibition of the PLN response was also dependent on time of sIL-1R administration, with treatment starting 1 day before exposure to allogeneic cells being the most effective (Fig. 1B). No significant inhibition was observed when treatment began 1 day after allogeneic cell challenge (Fig. 1B). Thus, sIL-1R appears to interfere with an early event in the induction of the lymphoproliferative response.

To determine whether the inhibitory effect of sIL-1R in the PLN system could be abrogated by simultaneous administration of exogenous IL-1, we treated recipients with a mixture of sIL-1R and IL-1α or with sIL-1R and IL-1α administered separately. Administration of IL-1α alone had no significant effect on the PLN response (Fig. 1C). However, when IL-1α was administered in conjunction with otherwise inhibitory doses of sIL-1R, the allogeneic PLN response was restored to that of MSA-treated controls (Fig. 1D). The complete reversal of the inhibitory effect of sIL-1R by IL-1 suggests that sIL-1R acts by virtue of its ability to neutralize IL-1.

We also examined cells obtained from alloreactive nodes for expression of T cell and B cell markers and for evidence of specific sensitization to the allogeneic cells used to induce the response. Although the initiation of the PLN response depends on the presence of T cells in the host (*16*), the actual cellular increase in the node occurs in both T cell and B cell compartments (Table 2). Among T cells, both $CD4^+$ and $CD8^+$ cells are affected. The sIL-1R treatment interfered with increases in all lymphoid subsets in the draining lymph node as defined by these markers.

We determined whether T cells were sensitized to alloantigens in the lymph nodes of sIL-1R–treated mice by testing lymph node cell populations for their capacity to respond in mixed leukocyte culture to the specific allogeneic cells used for sensitization and, as

controls, to syngeneic and third party allogeneic cells. Cells from BALB/c PLNs draining the C57BL/6 cell injection site proliferated to a higher degree in response to in vitro challenge with the immunizing C57BL/6 cells than did (i) cells obtained from nodes of the same mice that drain the site of injection of syngeneic cells or (ii) cells from unprimed mice (Table 3). The secondary-type response, characteristic of sensitized T cells, occurred regardless of whether mice were injected with sIL-1R or MSA. The response was specific for cells from the C57BL/6 immunizing strain, as the response of the same cells to third party SJL stimulating cells was almost identical to the primary response of cells from nonimmunized mice (Table 3). Thus, although sIL-1R administration significantly decreases the size of lymph nodes draining the allogeneic cell injection site, the cell populations that are present in such nodes contain specifically sensitized cells. Further experimentation will be necessary to determine whether the absolute number of sensitized cells present in the nodes has been altered by sIL-1R treatment. Most simply interpreted, the data indicate that sIL-1R may modulate the allogeneic response in vivo not by interfering with T cell recognition and sensitization, but perhaps by perturbing subsequent processes, such as secretion of other cytokines that normally serve to amplify the immune response. In this regard, allograft survival can be prolonged by administration of antibodies to the IL-2 receptor (*14, 15*) or antibodies to γ-interferon (*17*). Alternatively, sIL-1R may block allograft rejection independently of T cell involvement, by precluding induction of the inflammatory events normally triggered by IL-1.

Soluble IL-1R can modulate allograft survival and allo-induced lymphoproliferation in vivo; thus, IL-1 is probably an important mediator of alloreactivity. Support for this idea comes from recent studies showing that antibodies to IL-1α or to the IL-1 receptor also inhibit allo-induced PLN hypertrophy in vivo (*18*).

These results demonstrate the ability of a soluble cytokine receptor to modulate biological activity upon exogeneous administration in vivo, presumably by acting as a neutralizing agent for the endogenously produced, corresponding ligand (IL-1), and provide evidence suggestive of the therapeutic potential of soluble cytokine receptors in a variety of clinical disorders.

Table 2. Effect of sIL-1R administration on the surface phenotype of lymph node populations. BALB/c mice were injected in one footpad with syngeneic spleen cells and in the contralateral footpad with allogeneic (C57BL/6) spleen cells that had been irradiated (2500 rads) before injection. On days −1, 0, and +1 relative to spleen cell injection, mice were given daily intraperitoneal injections of sIL-1R (2 μg) mixed with MSA (100 ng) or of MSA alone. Cell suspensions were prepared from the draining lymph nodes and resuspended in phosphate-buffered saline (PBS) containing 1% fetal bovine serum (FBS) and 0.1% NaN_3 for flow cytometric analysis. For antibody binding, 0.5×10^6 to 1×10^6 cells were incubated with optimal concentrations of antibody for 30 min at 4°C and then washed three times. The reagents utilized were monoclonal antibody (MAb) GK1.5 (anti-CD4) conjugated to phycoerythrin and MAb 53-6 (anti-CD8) conjugated to fluorescein isothiocyanate (Becton Dickinson). Affinity-purified goat $F(ab')_2$ to immunoglobulin M (IgM) (μ chain–specific, Tago) was utilized to detect B cells. Single color flow cytometry was done with a single laser FACScan (Becton Dickinson). Cells not incubated with fluorochrome-conjugated antibodies were analyzed to determine light scattering characteristics and autofluorescence levels.

Treatment group	Cells injected*	Cells per node × 10^{-6}			Total cellularity
		$CD4^+$	$CD8^+$	IgM^+	
MSA	Syngeneic	0.80	0.21	0.32	1.5
sIL-1R	Syngeneic	1.45	0.48	0.68	2.6
MSA	Allogeneic	2.85	0.88	5.25	9.4
		3.00	0.76	3.90	8.3
sIL-1R	Allogeneic	1.28	0.31	1.11	3.0
		1.46	0.37	1.18	3.3

*Nodes draining the site of syngeneic cell injection were pooled from two mice. Data for the nodes draining the site of allogeneic cell injection are from individual mice. This is a representative experiment of three performed. Similar increases in allogeneic node cellularity compared to that in syngeneic nodes were observed for both treatment groups in the other experiments. The average cellularity of syngeneic nodes harvested from sIL-1R–treated mice (mean ± SD, 1.52 ± 0.60 × 10^6 per node; n = 18) did not differ significantly from the average cellularity of syngeneic nodes harvested from MSA-treated mice (1.35 ± 0.5 × 10^6 per node; n = 14).

Table 3. Effect of sIL-1R administration on in vivo priming to allogeneic cells in the draining lymph node. BALB/c mice were injected in one footpad with allogeneic (C57BL/6 or SJL) spleen cells and in the contralateral footpad pad with syngeneic (BALB/c) spleen cells that had been irradiated before injection. Recipients were treated with daily intraperitoneal injections of sIL-1R (2 μg) mixed with MSA (100 ng) or MSA alone on days −1, 0, and +1 relative to spleen cell injection. To determine whether cells in the lymph nodes were primed to allogeneic cells, we established mixed leukocyte cultures in 96-well microtiter plates by culturing 2×10^5 lymph node cells with 1×10^6 irradiated (2500 rads) C57BL/6, SJL, or BALB/c spleen cells in Dulbecco's modified Eagle's medium supplemented with 5% FBS, 5×10^{-5} M 2-mercaptoethanol and additional amino acids (*21*). Cultures were pulsed overnight with [^{3}H]thymidine on day 3, 5, or 7 after culture initiation. Maximal proliferation occurred on day 5 and those results are shown. Nodes challenged with allogeneic cells of a particular type (C57BL/6 or SJL) were pooled, as were nodes challenged with syngeneic cells, before cell harvest and culture. MSA-treated mice that received C57BL/6 or SJL spleen cells exhibited a PLN Δ value of 2.3 ± 0.2 and 2.3 ± 0.3 mg, respectively; sIL-1R–treated mice that received C57BL/6 cells exhibited a PLN Δ value of 0.3 ± 0.1 mg (see legend to Fig. 1). Values are means ± SD.

Treatment of BALB/c host	Stimulating cells		Radioactivity (cpm × 10^{-3})
	In vivo	In vitro	
MSA	C57BL/6	C57BL/6	82.6 ± 6.7
		BALB/c	1.2 ± 0.02
		SJL	34.1 ± 6.0
MSA	BALB/c	C57BL/6	43.3 ± 13.2
		BALB/c	1.6 ± 0.5
		SJL	31.7 ± 4.0
MSA	SJL	C57BL/6	44.8 ± 2.9
		BALB/c	1.3 ± 0.02
		SJL	76.1 ± 3.8
sIL-1R	C57BL/6	C57BL/6	110.5 ± 16.9
		BALB/c	1.3 ± 0.2
		SJL	42.9 ± 3.6
sIL-1R	BALB/c	C57BL/6	36.5 ± 5.3
		BALB/c	1.4 ± 0.4
		SJL	20.8 ± 1.4
None	None	C57BL/6	51.1 ± 4.8
		BALB/c	1.8 ± 0.5
		SJL	40.3 ± 6.2

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23. The indicated dose of sIL-1R was administered together with 100 ng of MSA by intraperitoneal injection on days −1 and 0 and by subcutaneous injection on days 1 and 2, relative to antigen administration, except where otherwise noted. Control mice received MSA but no IL-1R. Specific pathogen–free 8- to 12-week-old BALB/c (H-2^d) and C57BL/6 (H-2^b) mice were obtained from the Jackson Laboratory. MSA was obtained from Sigma and used at a concentration of 1 μg/ml in pyrogen-free saline. Recombinant human IL-1α was generated in *Escherichia coli* and purified to homogeneity as described [S. R. Kronheim *et al.*, *Bio/Technology* **4**, 1078 (1986)]. Recombinant soluble IL-1R was expressed in HeLa cells and purified as outlined (*11*). IL-1α and sIL-1R were diluted in MSA to the appropriate concentration before injection. These diluted cytokine–cytokine receptor preparations always resulted in less than 20 pg of lipopolysaccharide endotoxin being delivered per treatment as measured by the *Limulus* amoebocyte assay (M.A. Bioproducts).
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Phosphorylation of the Polymeric Immunoglobulin Receptor Required for Its Efficient Transcytosis

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The endosomal compartment of polarized epithelial cells is a major crossroads for membrane traffic. Proteins entering this compartment from the cell surface are sorted for transport to one of several destinations: recycling to the original cell surface, targeting to lysosomes for degradation, or transcytosis to the opposite surface. The polymeric immunoglobulin receptor (pIgR), which is normally transcytosed from the basolateral to the apical surface, was used as a model to dissect the signals that mediate this sorting event. When exogenous receptor was expressed in Madin-Darby Canine Kidney (MDCK) cells, it was shown that phosphorylation of pIgR at the serine residue at position 664 is required for efficient transcytosis. Replacement of this serine with alanine generated a receptor that is transcytosed only slowly, and appears to be recycled. Conversely, substitution with aspartic acid (which mimics the negative charge of the phosphate group) results in rapid transcytosis. It was concluded that phosphorylation is the signal that directs the pIgR from the endosome into the transcytotic pathway.

EPITHELIAL CELLS HAVE TWO SEPARATE plasma membrane domains, apical and basolateral, that are characterized by distinct protein compositions. In MDCK epithelial cells, approximately one-half of the cell surface is internalized per hour (*1, 2*), and sorting of proteins from the endosomal compartment to their appropriate cellular destinations is critical for the maintenance of cell polarity. From this compartment, most membrane proteins are either recycled to the original cell surface (for example, transferrin receptor) (*3*) or transported to lysosomes for degradation [for example, epidermal growth factor (EGF) receptor] (*4*). Certain proteins, however, are segregated into separate vesicles and transcytosed to the opposite cell surface, presumably because they contain sorting signals that direct them into this transcytotic pathway. The pIgR, which transports immunoglobulin A (IgA) and immunoglobulin M (IgM) across a variety of epithelia, is a useful model for the study of signals that determine transcytosis (*5, 6*). Newly synthesized pIgR is delivered from the trans Golgi network (TGN) to the basolateral cell surface where it can bind ligand. Receptor-ligand complexes (or receptor alone) are rapidly endocytosed and enter basolateral endosomes, where segregation into transcytotic vesicles takes place (*7*). These vesicles are then transported to the apical cell surface, where the extracellular domain of the receptor is proteolytically cleaved and released into secretions. This released receptor fragment is referred to as secretory component (SC).

The nature of the sorting signals that direct pIgR into the transcytotic pathway is not known. In rat liver, the pIgR has been shown to be phosphorylated on serine (*8*), and it is thought that this modification may be important in receptor function. To address this issue directly, we used MDCK cells that stably express cDNA encoding the rabbit pIgR (*9*). The exogenously expressed receptor functions in these cells as in vivo, transporting IgA from the basolateral to the apical surface (*9*). If receptor phosphorylation were important in mediating sorting, it should occur in these cells as well. Immunoprecipitation of pIgR from [^{32}P]P_i (inorganic phosphate)-labeled cells revealed this to be the case (Fig. 1, lane 1). Phosphopeptide mapping (Fig. 1, lane 2) localized most of the incorporated ^{32}P to a peptide containing 4 of the 11 cytoplasmic serines in the rabbit receptor (*10*). We then used site-directed mutagenesis to define the most membrane-proximal of these, Ser^{664}, as the primary phosphorylation site. Mutation of this serine to alanine, a nonphosphorylatable residue, resulted in an 84% reduction in ^{32}P incorporation into the mutant Ala^{664} receptor (*11*) (Fig. 1, lane 3) and a total loss of incorporation into the major phosphopeptide produced after treatment with V8 protease (lane 4). This site is not analogous to those defined for any of the known serine-threonine kinases (*12*).

To analyze the role of phosphorylation at this site in receptor sorting, we made an additional mutation, substituting aspartic acid for the target serine (Asp^{664} receptor). The negatively charged aspartate has been shown to mimic the effect of serine phosphorylation in regulation of the enzyme isocitrate dehydrogenase (*13*), suggesting that it is the charge of the phosphate group that mediates this process. To examine the effects of these mutations on receptor sorting, we assayed the efficiency of sorting of wild-type, Ala^{664}, and Asp^{664} receptors at each of three points in the receptor pathway: vectorial delivery from the TGN to the basolateral cell surface, endocytosis from the cell surface, and postendocytotic sorting from the endosome to other cellular destinations (*14*).

To analyze receptor delivery to the basolateral cell surface, we used a modification of the trypsin sensitivity assay devised by Mat-

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