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Immunology

Soluble interleukin 2 receptors are released from the cell surface of normal murine B lymphocytes stimulated with interleukin 5

(receptor modulation/lymphokines)

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ABSTRACT **Murine T and B lymphocytes can be induced to release soluble interleukin 2 receptors (IL2Rs). This receptor is believed to be a truncated form of the 55-kDa chain of the cell-membrane-associated receptor. It has been speculated that this receptor may play an important immunoregulatory role by binding to interleukin 2 (IL-2). We report here that interleukin 5 can induce normal murine B cells to release soluble IL2Rs. This extends our finding that interleukin 5 similarly can induce murine B cells to express functional cell-surface-associated IL2Rs. Two possible mechanisms of release of soluble IL2Rs have been suggested. Soluble IL2R could be synthesized as a secretory form of the receptor lacking the transmembrane domain or by cleavage of the extracellular domain of the cell-surface-associated IL2R at the cell surface. To investigate which mechanism was operative, we radioiodinated the cell surface of normal murine splenocytes that had been cultured for 1 day with Con A to stimulate the expression of cell-surface-associated IL2Rs and the release of soluble IL2Rs. Under the conditions used, radiolabeling of internal proteins was not apparent. Labeled cells were then recultured with Con A, conditioned medium was taken from replicate cultures at various times after radioiodination, and the specific radioactivity of released soluble IL2Rs was determined by ELISA and RIA. We demonstrate that the specific radioactivity and the kinetics of change of the specific radioactivity are consistent with the hypothesis that the soluble IL2Rs are derived from the cell-surface-associated IL2Rs rather than being released in a secretory form.**

Interleukin 2 (IL-2) is a T-cell-derived lymphokine that has been shown to exert its biological effects through interaction with specific cell-surface receptors (1). One chain of this receptor has been cloned and is a 55-kDa glycoprotein designated Tac. Tac is expressed on activated human T and B lymphocytes (1, 2). An equivalent molecule has been identified on the surface of activated murine lymphocytes (3, 4). Studies using an ELISA have demonstrated that this molecule can be released by T and B lymphocytes when appropriately stimulated (5, 6). Soluble IL-2 receptors (IL2Rs) have also been detected in normal human and murine sera (7–9), and increased levels have been identified in certain disease states such as transplant rejection (10), lymphomas, and acquired immunodeficiency syndrome (11). While the soluble IL2R remains only partially characterized, it has been established that the soluble IL2Rs can bind IL-2 with low affinity (M.S.L. and G.J.V.N., unpublished results; ref. 12). Such observations have led to the hypothesis that relatively high quantities of soluble IL2R may compete with high-affinity receptors for binding of free IL-2 (5). Speculation on the manner of release has occurred with two possible mechanisms having been proposed, proteolytic cleavage of the low-affinity receptor from the cell surface or release of a secreted receptor lacking the transmembrane domain. Much work has also addressed the cellular requirements for soluble IL2R production by both T and B cells. This work has centered on stimulating cells with either mitogens (6) or antigens (13).

We report here that normal murine splenocytes can be stimulated to release soluble IL2Rs in response to interleukin 5 (IL-5). This work extends our observations that B cells can be stimulated by IL-5 to express functional IL2Rs (3). To determine the mechanism of release, ELISA and RIA were used to establish that soluble IL2Rs released from the stimulated murine splenocytes that had been cell-surface-radioiodinated and then recultured with Con A had a similar specific radioactivity to IL2R on the surface of these cells before reculture. Radioiodination of internal proteins, as determined by radiolabeling of actin, was minimal under the radioiodination techniques employed. These results suggest that soluble IL2R is cleaved from the cell surface of activated cells, not released in a secretory form.

MATERIALS AND METHODS

Con A-Stimulated Murine Splenocyte Conditioned Medium (CM). Con A-stimulated murine splenocyte CM was prepared by culture of 10^6 normal CBA murine splenocytes with Con A at 2 μg/ml (Pharmacia) in 1 ml of RPMI 1640 medium supplemented with 5% (vol/vol) fetal calf serum (Flow Laboratories) and 10^{-4} M 2-mercaptoethanol for 3 days. Following harvest, the CM was concentrated by a factor of 10 with a stirred ultrafiltration cell (Amicon) and was used as a standard source of soluble IL2R for RIA and ELISA.

Antibodies. The rat monoclonal anti-IL2R antibody 7D4 was produced as ascites fluid in nude mice primed with pristane. The rat monoclonal anti-IL2R antibody PC61, which binds to the IL2R at an epitope distinct from 7D4 (14), was similarly produced as ascites fluid in a nude mouse. PC61 was purified by passage over a hydroxyapatite column supplied as part of the monoclonal antibodies purification system (Bio-Rad). Where indicated, purified PC61 that had been conjugated to biotin was used. The mouse monoclonal anti-fluorescein IgM antibody (FluIgM-1) has been described (15) and was produced as ascites fluid in nude mice primed with pristane. FluIgM-1 was a generous gift from A. Boyd (Walter and Eliza Hall Institute). The rat monoclonal anti-IL-5 antibody TB13 was produced as ascites fluid as described (16) and has been shown to inhibit the actions of IL-5. TB13 was a generous gift from K. Takatsu (Kumamoto University Medical School, Kumamoto, Japan).

Cells. Mice from the inbred strain CBA/CaH/WEHI were used as spleen donors at 8–10 weeks of age. Mice were held

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Abbreviations: IL2 and -5, interleukin 2 and 5, respectively; IL2R, IL-2 receptor; EL4-BGDF, EL4 B-cell growth and differentiation factor; CM, conditioned medium.

as specific-pathogen-free until shortly before they were killed. EL4 cells are a mouse thymoma line known to be IL2R-negative and were harvested 3 days after their last medium change.

Lymphokines. IL-5 was used as either CM from transfected COS cells or as IL-5 that had been purified by three HPLC steps (purified IL-5) (ref. 17; H. D. Campbell, C. J. S., Y. Wang, Y. Hort, M. E. Martinson, W. Q. J. Tucker, A. Stellwagen, M. Starth, and I. G. Young, unpublished data). Sham IL-5 refers to CM from nontransfected COS cells. EL4 B-cell growth and differentiation factor (EL4-BGDF) is a 10-fold concentrate of medium conditioned by Con A-stimulated EL4 thymoma cells as described (18) and was used as a source of T-cell-derived factors.

Preparation and Culture of Murine Splenocytes and Splenic B Cells. B cells were obtained by use of a complement-mediated cell lysis step as described (3). In brief, splenocytes were held on ice in the presence of the monoclonal antibodies anti-Thy-1.2 and anti-Mac1. Newborn rabbit complement was then added, and cells were maintained at 37°C for 20 min. After two rounds of this procedure, cells were subjected to density separation in a metrizamide density gradient (Nyegaard, Oslo). Live cells were stained for surface IgM with fluorescein-labeled goat anti-mouse IGM (μ heavy chain specific antibody) (Southern Biotechnology Associates, Birmingham, AL) and analyzed with a fluorescence-activated cell sorter (FACS II, Becton Dickinson). Approximately 90% of the cells were surface IgM-positive, and <1% were Thy-1.2-positive, whereas after 3 days of culture with EL4-BGDF, >99% were surface IgM-positive, and <1% were Thy-1.2-positive. In addition, fractionated cells were found to be unresponsive to Con A stimulation. These cells enriched for B cells are referred to as B cells throughout this paper and were cultured at 2.5×10^5 cells per 200-μl 96-well culture plate (Disposable Products, Adelaide, Australia) in 150 μl of RPMI 1640 supplemented with 5% (vol/vol) fetal calf serum, 10^{-4} M 2-mercaptoethanol, and various lymphokines as indicated.

Assessment of Soluble IL2R Release. Soluble IL2R release was assessed by ELISA. Briefly, 96-well ELISA plates (Dynatech, Alexandria, VA) were coated overnight with 60 μl of 7D4 diluted in carbonate buffer (pH 9.6) to $\approx$1 μg/ml. All reactions were performed at room temperature, and all coatings subsequent to 7D4 were performed in 0.3% nonfat milk powder/0.05% Tween 20/1% fetal calf serum in mouse tonicity phosphate-buffered solution (MTPBS; ref. 3). Following this initial coating, trays were washed, and 50 μl of sample CM was added in five serial dilutions and incubated for 4 hr. Plates were then washed again, and 50 μl of biotinylated PC61 at 4 μg/ml was added. After a subsequent 4 hr, plates were washed, and 50 μl of avidin-conjugated horseradish perioxidase (Vector Laboratories, Burlingame, CA) was added at 15 μg/ml for another 4 hr. Plates were then washed and developed with the substrate 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma), and the absorbance of the fluid in the wells was read 1 hr later with a Titertek Multiskan MC (Flow Laboratories) with dual wavelengths (414 nm and 492 nm). The concentration of soluble IL2R in sample CM was measured by comparison of results from a titration of each sample against a standard source of soluble IL2R. The standard source of soluble IL2R used was Con A-stimulated murine splenocyte CM, and for this purpose a full titration curve of Con A-stimulated murine splenocyte CM was done for each assay tray. The concentration of soluble IL2R in Con A-stimulated murine splenocyte CM had been measured by use of a modified RIA with Scatchard analysis (M.S.L. and G.J.V.N., unpublished results).

Cell Radioiodination and RIA of Cell CM. Splenocytes were cultured for 1 day with 2.5×10^5 cells per 200-μl 96-well culture plate (Disposable Products) in 150 μl of RPMI 1640 (Flow Laboratories) supplemented with 5% (vol/vol) fetal calf serum, 10^{-4} M 2-mercaptoethanol, and Con A at 2.5 μg/ml. These splenocytes or EL4 cells, prepared as described, were then harvested, washed three times with MTPBS, and resuspended at 10^7 cells per 70 μl. To this volume of cells was added 50 μl of Enzymobeads coated with lactoperoxidase and glucose oxidase (Bio-Rad) rehydrated in pure water, 25 μl of 1% β-D-glucose in MTPBS, and 0.5 mCi of $Na^{125}I$ in 5 μl (1 Ci = 37 GBq; Amersham). This mixture was incubated at room temperature for 30 min, viability was >85%, and incorporation of ^{125}I was >30%. Cells were then washed twice in MTPBS, resuspended in RPMI 1640 supplemented with fetal calf serum, 2-mercaptoethanol, and Con A, and cultured at 2×10^6 cells per well in 150 μl of medium as above. CM (110 μl) was removed from duplicate wells at the times indicated. This CM was stored frozen and assayed for soluble IL2R content with an ELISA as described. At the same time, the CM was split, and, as a control, 50 μl of a 1:2 dilution of the CM was added to an ELISA well coated with ascites fluid containing the irrelevant anti-fluorescein monoclonal antibody FluIgM-1 diluted in carbonate buffer (pH 9.6) at the same concentration as for 7D4 in the ELISA for soluble IL2R. After reading the ELISA plate, the reaction buffer was poured off with a flick, and radioactivity in individual wells was measured for 5 min with a Crystal multidetector RIA system (United Technologies Packard, Downers Grove, IL). Specific binding of radioiodinated soluble IL2R was defined as the difference between those cpm recorded for the sample incubated with FluIgM-1 ascites fluid-coated wells (nonspecific binding) and cpm recorded for the same sample incubated with 7D4 ascites fluid-coated wells (total binding). The specific radioactivity of the bound soluble IL2R was then calculated by multiplying the cpm by the ng of soluble IL2R actually bound to the plate as measured by the ELISA. After iodination, an aliquot of 2×10^6 cells was lysed in lysis buffer [10 mM Tris·HCl (pH 7.4), 0.15 M NaCl, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, chymostatin at 1 μg/ml, leupeptin at 1 μg/ml, antipain at 1 μg/ml, and pepstatin at 1 μg/ml (Sigma)]. After lysis for 1 hr on ice, each sample was centrifuged for 20 min at $16,000 \times g$ to remove nuclei and other unlysed organelles. Each lysis volume was then diluted 1:1 with 0.46% $NaDodSO_4$ and submitted to $NaDodSO_4$/PAGE [10% (wt/vol) acrylamide]. After electrophoresis the gel was stained with Coomassie blue and submitted to autoradiography for 14 hr with a Hyperfilm-MP autoradiography film (Amersham).

RESULTS

Lymphokine Control of Soluble IL2R Release from Normal Murine B Cells. To investigate the requirements of normal murine B cells for soluble IL2R production, B cells were cultured in medium alone or medium supplemented with 20% (vol/vol) EL4-BGDF, 1% IL-5, or 1% sham IL-5 over 7 days. On each day, CM was collected from duplicate wells and assayed for soluble IL2R content. At the same time the number of live cells per well was determined by counting eosin-excluding cells with a hemocytometer. Assuming a molecular mass of 45 kDa for soluble IL2R (5), the number of receptors released per live cell per hr over the preceding day of culture was calculated (Tables 1 and 2). EL4-BGDF and IL-5, but not medium alone or sham IL-5, were able to induce the generation of soluble IL2R. B cells were also cultured with various concentrations of either EL4-BGDF or purified IL-5. After 5 days in culture, CM was collected from duplicate wells and assayed for soluble IL2R content (Fig. 1). B cells cultured with lipopolysaccharide with or without 1% purified IL-5 incorporated similar amounts of [^{3}H]thy-

Table 1. Kinetics of soluble IL2R generation by B cells cultured in medium alone or medium supplemented with EL4-BGDF

Time in culture, days	Change in soluble IL2R, pg/150 μl		Live cells, no. × 10^{-5} per well	Receptors, no. per cell per hr*
	Medium alone	+ EL4-BGDF		
1	<2	12.8	2.03	35
2	<2	3.1	2.42	7
3	<2	39.9	3.34	66
4	1	47.6	3.29	80
5	3	35.9	2.15	93
6	5	25.9	1.90	75
7	—	−2.7	0.83	—

Cultures contained 2.5 × 10^5 cells per well, and CM and cells from quadruplicate wells were collected daily. For live cell counts and determination of number of receptors, cells were cultured with EL4-BGDF. Cells were counted in the presence of eosin to determine the number of live cells per well. ELISA for soluble IL2R in CM was performed in quadruplicate, and the mean increase in soluble IL2R as pg per well was determined.

*Average number of receptors released per live cell per hr over the preceding 24 hr, assuming a molecular mass of 45 kDa for the soluble IL2R.

midine over a 3.5-hr period, indicating that purified IL-5 itself was not toxic to cells (data not shown). A dose-dependent increase in the number of soluble IL2Rs generated with various concentrations of either EL4-BGDF or purified IL-5 was noted. Whereas both were able to induce soluble IL2R release, EL4-BGDF stimulation consistently induced the release of more receptors per cell than did purified IL-5 at plateau levels. This was possibly due to the presence of other soluble IL2R-inducing activities within EL4-BGDF.

Soluble IL2R Release by EL4-BGDF Is IL-5 Dependent. Given that we have shown (3) that IL-5 is the main activity present in EL4-BGDF capable of inducing cell-surface-associated IL2Rs in B cells, we decided to investigate whether this was true for the induction of soluble IL2Rs. B cells were cultured with 20% (vol/vol) EL4-BGDF and various concentrations of TB13, an anti-IL-5 monoclonal antibody. After 5 days CM from replicate wells was collected and assayed for soluble IL2R content (Table 3). B cells cultured with lipopolysaccharide with or without TB13 incorporated similar accounts of [^{3}H]thymidine over a 1.5-hr

Table 2. Kinetics of soluble IL2R generation by B cells cultured in medium supplemented with sham IL-5 or IL-5

Time in culture, days	Change in soluble IL2R, pg/150 μl		Live cells, no. × 10^{-5} per well	Receptors, no. per cell per hr*
	Sham IL-5	IL-5		
1	<2	<2	3.2	—
2	<2	<2	2.4	—
3	<2	10	2.4	23
4	<2	0	1.6	—
5	<2	6	2.7	12
6	6	3	1.9	9
7	−4	2	1.3	7

Cultures contained 2.5 × 10^5 cells per well, and CM and cells from replicate wells were collected daily. For live cell counts and determination of receptor numbers, cells were cultured with 1% IL-5. Cells were counted in the presence of eosin to determine the number of live cells per well. ELISA for soluble IL2R was performed on duplicate CM samples, and the mean increase in soluble IL2R as pg per well was determined.

*Average number of receptors released per cell per hr over the preceding 24 hr.

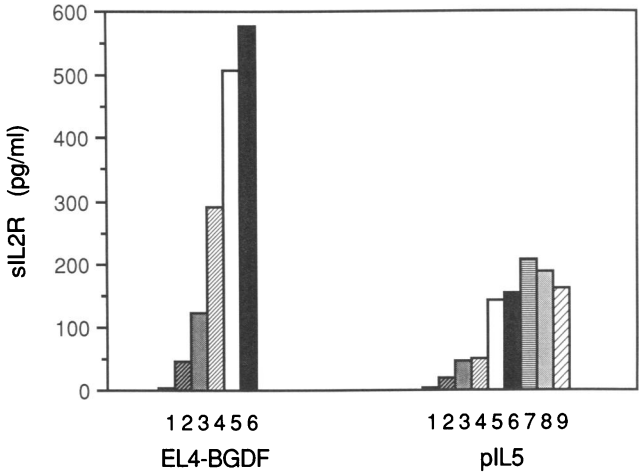

FIG. 1. B cells were cultured with various concentrations of EL4-BGDF or purified IL-5 (pIL-5) for 5 days, and CM was taken from duplicate wells for soluble IL2R (sIL2R) assay. Bars represent the mean of the sIL2R content of the duplicate wells. Concentrations of EL4-BGDF used per culture were medium alone (bar 1), 1% (vol/vol) (bar 2), 5% (vol/vol) (bar 3), 10% (vol/vol) (bar 4), 15% (vol/vol) (bar 5), and 20% (vol/vol) (bar 6). Concentrations of purified IL-5 used were medium alone (bar 1), 0.01% (bar 2), 0.03% (bar 3), 0.05% (bar 4), 0.1% (bar 5), 0.2% (bar 6), 0.3% (bar 7), 0.5% (bar 8), and 1.0% (bar 9).

period, indicating that the antibody preparation itself was not toxic to cells (data not shown). Whereas TB13 did decrease the number of soluble IL2Rs generated in these cultures, there was still a significant number of soluble IL2Rs generated even at high concentrations of the antibody.

Selective Labeling of Cell-Surface Proteins by Radioiodination. To determine whether the lactoperoxidase radioiodination method used labeled internal, as well as cell-surface, proteins, cells were iodinated, and proteins were examined for incorporated label. After each iodination an aliquot of cells was lysed immediately and submitted to $NaDodSO_4$/PAGE and autoradiography. Although a prominent 43-kDa protein band (the molecular mass of the abundant intracellular protein actin) was found on Coomassie blue staining, only a very faint band was seen on the autoradiograph (Fig. 2). This contrasts with the heavily labeled bands found on autoradiographs for some presumably cell-surface proteins that stained only lightly or not at all with Coomassie. The degree of light labeling noted with actin is consistent with labeling of actin in the 5–10% of the cells that were dead and provides good evidence that under the conditions used radioiodination for live cells was selective for cell-surface proteins.

Measurement of the Soluble IL2R Specific Radioactivity. To investigate whether the soluble IL2R detected in the CM of Con A-stimulated splenocytes was cleaved from the cell surface or released in secretory form by the cells, normal

Table 3. Soluble IL2R from B cells cultured with EL4-BGDF and TB13

% TB13	Soluble IL2R, pg/ml
0	228
0.1	137
0.5	119
1.0	113

Cells were cultured with 20% (vol/vol) EL4-BGDF and various concentrations of TB13 (an anti-IL-5 monoclonal antibody) for 5 days. Values are means of the concentrations of soluble IL2R in the CM from two wells.

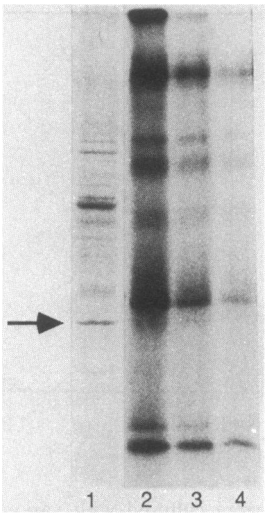

FIG. 2. Lysate of radioiodinated cells was dissolved in NaDodSO$_4$ and electrophoresed on a 10% polyacrylamide gel. The gel was then stained with Coomassie (lane 1) and exposed for autoradiograph for 14 hr (lanes 2, 3, and 4). The cell lysate was undiluted (lane 2), diluted 1:2 (lane 3), and diluted 1:4 (lane 4). The position of actin is marked by an arrow.

murine splenocytes that had been stimulated for 1 day with Con A (conditions known to induce both cell-surface IL2R expression and soluble IL2R secretion) were radioiodinated. After radioiodination cells were either recultured at 10^6 cells per well in 150 μl of medium supplemented with Con A or lysed in lysis buffer containing protease inhibitors. Lysis samples and CM samples taken from cultures at various times were analyzed for soluble IL2R content, and the specific radioactivity of the soluble IL2R was determined (Table 4). The specific radioactivity of the soluble IL2R released over the first 17 hr was considerably higher than that in the cell lysate containing intracellular (unlabeled) and cell-surface (labeled) soluble IL2Rs. With increasing cell proliferation over the next 2 days and presumably with increasing *de novo* cell-surface-associated IL2R production, the specific radioactivity of the released soluble IL2R decreased markedly. No soluble IL2R, and no specifically bound radioactivity, was detected in CM from radioiodinated EL4 cells in three experiments.

Table 4. Specific radioactivity of soluble IL2R released from radioiodinated cells

Sample	Soluble IL2R released from stimulated splenocytes		Specific radioactivity of released soluble IL2R
	pg/ml	cpm/ng	cpm/ng
Cell lysate	1356/1720	20,400/4802	—
CM			
0 hr	<40*/<40	<40 /<40	—
17 hr	437/895	63,600/6190	63,050/4530
	492/919	62,500/2870	
25 hr	766/1197	47,100/6950	12,867/3380
	762/986	39,500/5990	
72 hr	979/1703	37,700/5050	9,800/720
	976/1633	34,300/3750	

Con A-stimulated splenocytes or EL4 cells were radioiodinated and either lysed immediately or recultured with Con A. CM was withdrawn from cultures at various times as indicated, and the soluble IL2R content and specific radioactivity were assayed. Two typical experiments are shown for Con A-stimulated splenocytes. No soluble IL2R and no specifically bound radioactivity were detected in the CM from EL4 cells. The specific radioactivity (cpm/ng) of the soluble IL2R released between consecutive time points was calculated by multiplying the change in the soluble IL2R content in wells by the increase in cpm specifically bound.
*Less than detectable.

DISCUSSION

We report here that IL-5 and EL4-BGDF can induce the release of soluble IL2R from normal murine splenic B cells. We demonstrated that the soluble IL2R-inducing activity of EL4-BGDF can be partially abrogated by the anti-IL-5 monoclonal antibody TB13. This finding supports our results (3) that IL-5 and EL4-BGDF can induce cell-surface IL2R expression by otherwise unstimulated normal B cells. In those studies we demonstrated that the inducing activity in EL4-BGDF was probably IL-5. It is interesting then that TB13 was not completely able to inhibit the action of EL4-BGDF in the induction of soluble IL2R. Whereas it is possible that the concentrations of TB13 used were suboptimal and that at higher concentrations it would be able to completely inhibit this activity, this seems unlikely as a plateau level of inhibition appears to have been reached. The possibility remains that one or more other activities present in EL4-BGDF, distinct from IL-5, are capable of inducing B cells to release soluble IL2R.

Although it is known that activated T and B cells can release soluble IL2R *in vivo* and *in vitro*, the mechanism of release has remained unclear. The released IL2R has been shown by NaDodSO$_4$/PAGE molecular mass determination to be $\approx$10 kDa lighter than the cell-surface-associated IL2R while retaining all tested epitopes (5). Two mechanisms of release have been proposed. IL2R could be translated as a transmembrane-domain-deficient protein and released from the cell. This could arise from transcription from a separate gene lacking the transmembrane coding region or from alternate splicing of normal full-length mRNA producing a truncated mRNA. An alternative mechanism is that cell-surface-associated IL2Rs are cleaved from the cell surface. Both of these mechanisms of generating a soluble IL2R have been demonstrated before, for example, with secretory immunoglobulin (19), the insulin receptor (20), and class I major histocompatibility complex antigens (21).

Circumstantial evidence has suggested that cleavage of the soluble IL2R from the cell surface is the most likely mechanism of release. An increase in the serum level of soluble IL2R has been demonstrated in patients within 15 min of intravenous injection of IL-2 (22). It seems that cleavage of the IL2R from the cell surface would be more likely to occur in a 15-min period than transcription, translation, and release of a transmembrane-domain-deficient soluble IL2R. Furthermore, no cDNA clone corresponding to mRNA encoding a transmembrane-domain-deficient receptor has yet been isolated, and a selective susceptibility of the low-affinity receptor to Pronase has been noted (23). In the present study we found that soluble IL2R released from cell-surface-radioiodinated cells was radiolabeled to a specific radioactivity similar to cell-surface IL2Rs assayed immediately after radioiodination. This suggests that cleavage of IL2R from the cell surface is the mechanism of soluble IL2R release. We expected that the specific radioactivity of the soluble IL2R from cell lysates would be less than that of soluble IL2R released into the CM during the first 17 hr after radioiodination, as more than the expected amount of soluble IL2R was found when activated cells were lysed (8). This is probably due to the release of unlabeled intracellular IL2R that decreases the specific radioactivity of the total soluble IL2R in the lysate. The kinetics of change in the specific radioactivity of the released IL2R is also consistent with the progressive decrease in the specific radioactivity of the released IL2R from *de novo* IL2R synthesis associated with the marked cellular proliferation observed.

Given that soluble IL2R is cleaved from the cell surface, two mechanisms of release appear possible. The cell-surface IL2R could be cleaved from the cell close to the junction of the extracellular and transmembrane domains by a site-specific protease. The observation that the low-affinity, but not the high-affinity, IL2Rs are Pronase-sensitive would appear to support this hypothesis. A second possible mechanism for the cleavage of IL2R would be for post-translational linkage of IL2R through phosphatidylinositol to the cell membrane with subsequent cleavage of the receptor from the cell surface with phospholipase C. Such a mechanism of anchorage and release has been demonstrated with Thy-1 (24) and several other cell-surface proteins (25).

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