[Frontiers in Bioscience 10, 1462-1474, May 1, 2005]

THE ROLE OF INTERLEUKIN-2 RECEPTOR ALPHA IN CANCER

Deborah J. Kuhn and Q. Ping Dou

The Prevention Program, Barbara Ann Karmanos Cancer Institute, and Department of Pathology, School of Medicine, Wayne State University, Detroit, Michigan, USA.

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. IL-2Ralpha's Role in Cancer
- *3.1. Hematopoietic cancers*
  - *3.1.1. Adult T-cell leukemia (ATL)*
  - *3.1.2. Acute lymphoblastic leukemia (ALL)*
  - *3.1.3 B-cell chronic lymphocytic leukemia (B-CLL)*
  - *3.1.4 Hairy cell leukemia (HCL)*
- *3.2. Solid tumors*
  - *3.2.1. Colorectal cancer*
  - *3.2.2. Ovarian cancer*
  - *3.2.3. Prostate cancer*
  - *3.2.4. Melanoma*
  - *3.2.5. Lung cancer*
  - *3.2.6. Breast cancer*
  - *3.2.7. Others*
- *3.3. Cell culture models*
  - *3.3.1. IL-2Ralpha transfectants*
  - *3.3.2. Molecular characteristics*

4. Monoclonal Antibodies against IL-2Ralpha
- *4.1. 7G7/B6*
- *4.2. Murine anti-Tac (MAT)*
- *4.3. Humanized anti-Tac (HAT; daclizumab; Zenepax)*

5. Potential Use of Daclizumab as an Anticancer Drug
- *5.1. Effects on proliferation and cell cycle*
- *5.2. Effects on regulators of apoptosis*
- *5.3.mAbs combined with other chemotherapeutics*
  - *5.3.1.Toxins*
  - *5.3.2.Radiotherapy*
  - *5.3.3.Small molecule drugs*

6. Conclusion and Future Directions
7. Acknowledgments
8. References

1. ABSTRACT

Interleukin-2 (IL-2) is the major growth factor for activated T-lymphocytes and stimulates clonal expansion and maturation of these lymphocytes. IL-2 binds to its receptor complex, IL-2Ralpha, beta, and gamma chains, and exerts its effect *via* second messengers, mainly tyrosine kinases, which ultimately stimulate gene expression. The heterotrimerization of the receptor chains leads to high affinity binding for IL-2. The functional importance of IL-2Ralpha in hematopoietic cell systems is well known. However, the potential role that IL-2Ralpha plays in tumorigenesis is still not fully elucidated. Il-2Ralpha expression has been found in many types of cancers, including leukemia, lymphoma, lung, breast, head-and-neck and prostate. Recent evidence shows that high expression of IL-2Ralpha in tumors correlates with a poor prognosis for the patient. This review details the current known functions of IL-2Ralpha in cancer cells and some of the therapies used to combat IL-2Ralpha-mediated cell signaling.

2. INTRODUCTION

Interleukin-2 (IL-2), first described in 1976, was found to be a cytokine capable of sustaining the proliferative potential of T-lymphocytes (1, 2). Subsequently, IL-2 was determined to be the primary growth factor in activated T-lymphocytes, which is capable of driving clonal expansion and effector cell maturation (3, 4). IL-2 stimulation can also lead to the growth of natural killer (NK) cells (5). IL-2 mediates its biologic effects *via* the IL-2 receptor (IL-2R) complex. IL-2R is comprised of three distinct subunits: alpha, beta, and gamma chains (6,

7). IL-2Rbeta and gamma are involved in several cytokine-signaling pathways including IL-4, IL-7, IL-9, IL-15 and IL-21 (8-11). Both IL-2Rbeta and gamma chains are members of the hematopoietic superfamily of interleukin receptors, they possess cytoplasmic domains necessary for signaling, and their heterodimerization leads to an intermediate binding affinity (Kd = 10^{-9} M) for IL-2 (3, 12). The alpha chain (CD25+, Tac, p55) is involved in IL-2 and IL-15 signaling (8) and has a short cytoplasmic tail (13-residues) that is incapable of transducing signals downstream (13). Although IL-2Ralpha lacks the ability to transduce signals, its non-covalent binding with beta and gamma leads to an overall high binding affinity of the ligand (Kd = 10^{-11} M) (3, 12).

The traditionally studied role of IL-2R is related to proliferation of activated CD4-, CD8-, CD4+8+, CD4+, and CD8+ T cells. The importance of the IL-2Ralpha chain is demonstrated by its high affinity for IL-2. Although signal transduction after ligand stimulation can occur in the absence of IL-2Ralpha, when the alpha receptor is heterotrimerized to the beta and gamma receptor complex, the affinity for ligand increases 100-fold, leading to amplification of the signal (3). IL-2Rbeta and gamma subunits require phosphorylation in order to stimulate several signal transduction pathways because they lack intrinsic catalytic activity (14). Interaction of the Janus kinase (Jak) family with both the beta and gamma chains has been most extensively studied (15). For instance, IL-2 stimulation leads to binding of Jak3 to the gamma chain, phosphorylation of the gamma chain, and the subsequent phosphorylation of tyrosine kinase residues on the beta chain by the gamma chain (15-17). These newly phosphorylated sites on the beta chain act as docking sites for the signal transducers and activators of transcription (Stat) proteins, which then either homo- or heterodimerize and translocate to the nucleus to induce gene transcription (18). This is a common occurrence in Stat activation *via* cytokine receptors.

Other signaling pathways involving IL-2R include Jak3-dependent activation of Jak1, which has been shown to be the key proliferative signal in fibroblasts (19). In fact, naturally occurring point mutations in Jak3 that disrupt binding to the IL-2R results in severe combined immunodeficiency (SCID) (19). However, signaling can still occur in the absence of Jak3. The src-related kinase lck is activated upon intermediate affinity receptor dimerization and leads to anti-apoptotic signaling *via* the PI3K pathway, totally independent of Jak3 (20). Activation of Ras has also shown to be upregulated upon IL-2 stimulation in T cells. Deletion mutants of the IL-2Rbeta at the domain where lck associates leads to decreased Ras activity and decreased c-fos and c-jun induction (21). This has been linked to the phosphorylation of Shc protein by the Y-388 site located on the IL-2Rbeta (22). Another pathway for activation of Shc interacts with the adapter protein Grb-2, which associates with SOS, leading to Ras-Raf-p42/44MAPK activation (23-25).

Other studies indicate that IL-2Rbeta/gamma heterodimers can signal to activate Ras/Raf/Mek/Erk- and PI3K/Akt-mediated anti-apoptotic survival pathways without IL-2Ralpha binding (26) and also mediate Jak1-/Jak3-independent Bcl-2 induction (27). Furthermore, human primary intestinal epithelial cells and some human tumor epithelial cell lines (such as Caco-2, HT-29) were shown to express transcripts for both IL-2 receptor beta and gamma chains, and the receptors for IL-2 on intestinal epithelial cells lines appeared to be functional since IL-2 stimulation caused a rapid tyrosine phosphorylation of proteins (28). However, the role of IL-2Ralpha in these signal transduction pathways remains unclear. Even though the IL-2Rbeta/gamma complex is fully competent to signal, it appears that only the IL-2Ralpha/beta/gamma complex is the physiologically relevant form of IL-2R, because mice lacking IL-2Ralpha are phenotypically indistinct to IL-2-deficient mice (29-31). The critical role of IL-2Ralpha in IL-2-mediated signaling can be clearly shown by the evidence pointing to its role in increasing the affinity of the receptor to IL-2 as well as the aiding in the assembly of the functional receptor. A study utilizing the C-terminus domains of alpha, beta and gamma chains shows that assembly of all the domains occurs readily in the presence of the alpha chain, whereas assembly of beta and gamma chains was difficult to achieve and required prolonged exposure to IL-2 (32).

The stoichiometry of the IL-2 receptor has recently come to light. Vamosi et al., have shown that IL-2Rbeta/gamma/alpha and IL-15Ralpha chains form supramolecular receptor clusters in lipid rafts in a T-cell leukemic cell line (33). By using fluorescence resonance energy transfer (FRET) and confocal microscopy techniques, they determined that these receptors were co-expressed in the lipid rafts, even in the absence of IL-2 (33). Since IL-2 and IL-15 share the beta and gamma chains, it is not surprising that they have overlap in signaling functions. However, they do diverge in functionality in the case of activation-induced cell death (AICD). IL-2 signaling provides the elimination of self-reactive T-cells, while IL-15 prevents AICD activation (17). Until recently, it was unclear how the structurally similar IL-2 and IL-15 receptors can result in distinct biological immune responses upon ligand stimulation. It was found that IL-2Ralpha and IL-15Ralpha form homodimers/oligomers, which suggests a switching mechanism for the IL-2Rbeta/gamma heterodimer complex depending upon either IL-2 or IL-15 ligand stimulation (33).

Although there is a large amount of literature on the functionality of IL-2R in hematologic cell systems, the role that IL-2R plays in tumorigenic cells is not well characterized. Several studies have found overexpression of IL-2Ralpha in lung (34), skin (35), prostate (36), esophageal (37), and leukemic cancers (38-41). Additionally, the levels of soluble IL-2Ralpha (sIL-2Ralpha), a 45 kDa form shed from the cell surface, have been used as a prognostic indicator and its high levels in plasma correlate with poor survival rates in cancer patients (42-46).

3. IL-2RALPHA'S ROLE IN CANCER

The elevated expression of sIL-2Ralpha and IL-2Ralpha protein in tumor cells has been detected in a vast

array of cancers (34-46). This section will discuss the role that IL-2Ralpha plays in the growth and survival of neoplasias, including the diagnostic value of correlating IL-2Ralpha levels and the patients' response to treatment and survival. A study in 1990 identified 62 reports over a 5-year period that were based on identifying the clinical significance of utilizing sIL-2Ralpha in a variety of diseases, including T- and B-cell lymphoid cancers as well as autoimmune disorders (47). The authors concluded that sIL-2Ralpha levels are rapid and reliable indicators of both disease progression and prognosis (47). Additionally, elevated mRNA and protein expression of IL-2Ralpha has been identified in many solid tumor types, such as ovarian, lung, head-and-neck, and breast (see **Section 3.2**).

3.1. Hematopoietic cancers

3.1.1. Adult T-cell leukemia (ATL)

Adult T-cell leukemia or ATL occurs as a result of the infection by the human T-cell lymphotrophic virus-1 (HTLV-1) (48, 49). The disease is characterized by an overabundance of activated T-cells with IL-2Ralpha expression on their cell surface and consists of four stages: smoldering, chronic, lymphoma, and acute. It is not surprising that the levels of sIL-2Ralpha and/or expression of IL-2Ralpha protein can be used as indicators of activated T-cells. Araki *et al.*, showed that the mean values of sIL-2Ralpha were 788 U/ml for patients with the smoldering type, 1961 U/ml for the chronic type, and 9704 U/ml for the acute/lymphoma type of ATL (38). These levels matched well with the load of HTLV-1 infection and the pathophysiological status of disease. Additionally, four cell lines [K562, Molt4, Molt4/HTLV-III, and GH1 (HIV-2+)] that were not HTLV-1-infected were evaluated and all were negative for sIL-2R, whereas the HTLV-1-infected cell lines (MT1, MT2 and HUT102) produced sIL-2R levels of 1430 U/ml, 1920 U/ml and 8200 U/ml, respectively (38). Several other studies also point to the importance of IL-2Ralpha protein expression (50-52). Therefore, sIL-2Ralpha levels and IL-2Ralpha protein expression provide a useful measurement to assess disease status.

3.1.2. Acute lymphoblastic leukemia (ALL)

Over one-third of childhood cancers present as leukemia, and the most prevalent form, acute lymphoblastic leukemia, is linked with chromosomal aberrations. A study from St. Jude Children's Research Hospital revealed that sIL-2Ralpha serum levels (> 2,000 U/ml) in patients with non-T, non-B ALL is associated with a poorer outcome after treatment (39). Of the 344 children assessed, the levels of sIL-2Ralpha ranged from 267 to 80,000 U/ml, compared to the control population whose values ranged from 170 to 738 U/ml (39), providing additional evidence that sIL-2Ralpha levels can be used to assess disease status and outcome.

3.1.3 B-cell chronic lymphocytic leukemia (B-CLL)

B-CLL is the most predominant form of leukemia in the Western Hemisphere and is still regarded as an incurable disease (53). In a study conducted by Semenzato *et al*., peripheral blood samples from 54 patients with B-CLL were evaluated for sIL-2Ralpha/CD25 by an enzyme-linked immunosorbent assay (ELISA) (41). The B-CLL patient samples were compared to normal age-matched controls. In 51 B-CLL patients (94.4%), the average level of sIL-2Ralpha was 1,781 U/ml compared to the control population at 276 U/ml (41). Lower levels of IL-2Ralpha were correlated with less-invasive disease. Conversely, another study indicated that CD23 plays a more prominent role in B-CLL than CD25 (40). One hundred and five patients with early stage B-CLL were evaluated for both markers and 93 of those patients displayed high sIL-2Ralpha and CD23, which was associated with bone marrow infiltration and thymidine kinase levels. However, only CD23 was linked with disease progression of 76 of the untreated patients at initial diagnosis (40). Furthermore, several groups reported characterization of IL2 receptors in B-CLL cells, including Tsilivakos *et al*. (54) and Yagura *et al*. (55).

3.1.4 Hairy cell leukemia (HCL)

Hairy cell leukemia, or leukemic reticuloendotheliosis, is characterized by lymphocytic cells with cytoplasmic projections. These cells commonly infiltrate into the spleen and bone marrow (56). Relapse of this disease is common even with the use of the currently approved therapies, such as 2-chlorodeoxyadenosine and deoxycoformycin. Similar to the other hematopoietic malignancies previously described, HCL patients also exhibit high levels of sIL-2Ralpha (57-59). The median level detected in serum is 19,310 U/ml. Several hematologic parameters were assessed and decreased sIL-2R levels were correlated with a reduction in splenomegaly and a decreased number of marrow residing hairy cells (57-59). Recently, another study showed that reductions of sIL-2Ralpha correlated well with response to treatment with rituximab, a monoclonal antibody that targets CD20 antigen expression, in patients with relapsed or refractory HCL (60). In addition, several groups have investigated the role of IL-2R in acute myeloid leukemia (AML) cells (61-65).

3.2. Solid tumors

Much of the data regarding IL-2Ralpha and solid tumors concerns the penetrating lymphocytes into the tumorigenic site of origin. While these data are pertinent and can be used to assess tumor burden and stage, there are data that indicate that tumor tissues displays up-regulated IL-2Ralpha, especially tumors of the lung (34), prostate (36), melanoma (35), and breast (66). Recent *in vitro* data using stable transfectants of IL-2R revealed that over-expression of IL-2Ralpha in squamous cell carcinoma of the head and neck leads to increased proliferation, drug resistance, and transforming activity (67). Data regarding both the soluble form of IL-2Ralpha and cellular expression of IL-2Ralpha are included in this section. The biomolecular consequences of IL-2Ralpha overexpression will be discussed later in Section 3.3.

3.2.1. Colorectal cancer

IL-2Ralpha levels are a good prognostic indicator in colorectal cancer. Non-invasive means of measuring disease stage is an important focus for clinicians. Murakami *et al*., have shown that preoperative levels of sIL-2Ralpha correlate very well with several disease

factors, such as stage, lymph node metastasis, liver metastasis, grade, and immunosuppressive acidic protein (IAP) (45). These researchers found that the higher serum levels of IL-2Ralpha are indicative of Dukes stage C, while lower levels matched well with patients in Dukes stage A. Additionally, patients with metastasis to the lymph nodes also exhibited higher levels of sIL-2Ralpha than those without lymph node involvement (45). Another significant study showed a 95% probability that colorectal cancer is stage I or II, without infiltration to the lymph nodes when the serum levels of sIL-2Ralpha are less than 522 U/ml. Conversely, when the sIL-2Ralpha level is greater than 522 U/ml, there is a 95% probability that the disease is stage III with lymph node infiltration (44).

3.2.2. Ovarian cancer

A study conducted by Ferdeghini *et al.*, included 183 preoperative patients with ovarian masses and found that sIL-2Ralpha levels were elevated in patients with epithelial ovarian cancer compared to those with benign ovarian diseases, 79.6% *versus* 11.6% respectively (68). Another study conducted in 1994 focused on identifying the cellular source of sIL-2Ralpha in advanced epithelial ovarian cancer (43). Protein and mRNA levels for IL-2Ralpha were assessed in peripheral blood mononuclear cells (PBMC). Additionally, immunochemistry and flow cytometric analysis were performed on ascitic cell infiltrates and on primary and metastatic lesions. The study concluded that normal and malignant PBMC had little or no detectable mRNA or protein expression for IL-2Ralpha and very few cells were positive by immuochemistry and flow cytometric analysis (43). The cells that were positive were cytologically characterized as lymphocytes. Thus, the infiltrating lymphocytes were the main source of sIL-2Ralpha in ovarian cancer patients (43).

3.2.3. Prostate cancer

A recent study examined the presence of IL-2 receptors in normal prostates, benign prostatic hyperplasia (BPH), and prostatic cancer (PC) *via* ELISA, Western blot, and immunohistochemistry (36). All three types of specimens showed immumoreactivity with the antibodies for IL-2 receptors, but only the PC cells displayed a much higher immunoreaction than normal prostates (36). The author also determined that the location of IL-2R expression was in the epithelium of BPH prostates and that this expression was associated with high levels of the anti-apoptotic protein Bcl-2, which may account for the low apoptosis index related with this disease (36).

3.2.4. Melanoma

One of the most aggressive types of cancer is melanoma with survival rates ranging from a few months to a few years. Vuoristo *et al.*, found that there was a trend toward higher sIL-2Ralpha levels in patients with metastasis and sIL-2R seemed to serve as a marker of tumor load in metastatic melanoma (69). However, another study found that IL-2Ralpha protein was expressed in cultured melanoma cells (35). Furthermore, immunohistochemistry from cutaneous and ocular melanoma explants showed positive staining for IL2Ralpha in a high percentage of tumor cells (35). In addition, several other groups have studied the functions of IL-2 and its receptors in melanoma cells (70, 71). These data indicate that not only can serum sIL-2Ralpha be used as a prognostic indicator, but also melanoma cells can have the high affinity receptors for IL-2 (36).

3.2.5. Lung cancer

Lung and bronchial cancers account for an estimated 13% of all new cancers diagnosed annually and account for approximately 28% of all cancer deaths (72). Lung cancer is divided into two categories: small cell lung cancer (SCLC) and non-small lung cancer (NSCLC) (73). Two subtypes of NSCLC are adenocarcinoma and squamous cell carcinoma. A study from Ohio State University recently examined the gene expression profile changes in these SCLC and NSCLC and the gene expression differences between the adenocarcinomas and squamous cell carcinomas (34). They found 45 genes that were differentially expressed, by at least 2-fold, in tumor tissues *versus* normal tissues. One of the genes identified was IL-2Ralpha (34). Genes that had >50% differential expression were selected for further analysis and IL-2Ralpha was included in this category. A total of 7 lung tumors (3 adenocarcinomas, 4 squamous cell carcinomas) revealed increased levels of mRNA for IL-2Ralpha that correlated well with the cDNA expression array when compared to the adjacent normal tissues (34). An additional study pointed to the significance of sIL-2Ralpha levels in both SCLC and NSCLC patients and showed a very tight correlation in 62 lung cancer patients with sIL-2Ralpha level ($p < 0.01$) and relation to clinical stage, response to therapy, and patient survival time (74).

3.2.6. Breast cancer

A study in Japan exploring the feasibility of using sIL-2Ralpha in breast cancer patients for a prognostic indicator of distant metastasis showed that sIL-2Ralpha levels correlate well with the occurrence of metastasis (46). Thirty-seven preoperative breast cancer patients were investigated to correlate sIL-2Ralpha levels with several factors, including lymph node metastasis, distant metastasis, estrogen and progesterone receptor expression, tumor size, histopathological type, and stage grouping. The sIL-2Ralpha levels measured by ELISA determined that patients with stage III and IV breast cancer had significantly higher levels of sIL-2Ralpha than the normal controls (46). Furthermore, the sIL-2Ralpha levels in stage III and IV patients were higher than those with stage I and II breast cancer. Tumor volume is also a good prognostic indicator in breast cancer and this study found that patients with T3 and T4 stage disease had significantly higher levels of sIL-2Ralpha than those with T1 and T2 stage disease (46).

An immunohistochemical study on infiltrating ductal breast carcinomas found a high percentage of IL-2Ralpha expression (60%) and the expression of both the IL-2Rbeta and gamma chains (80%) (66). Total or partial mastectomy specimens from 52 women were used in this study and compared with the breast biopsies of 13 women with benign fibrocystic lesions. The study found that all three types of IL-2R were on both the cell surface and

cytoplasm in both types of the epithelial cells (66). Staining for IL-2Ralpha was found to be very weak in the benign fibrocystic lesions and intense in the infiltrating tumors. This data indicates that high expression of IL-2Ralpha may contribute to the development of tumors by promoting proliferation and/or inhibiting apoptosis (66).

3.2.7. Others

The expression of IL-2Ralpha has been described in a variety of other tissue types both normal and malignant, including intestinal epithelial cells (75), human embryonic fibroblasts (76), brain (77), renal (78), gastric (78), and pituitary cells (79). It is interesting to note that the IL-2Ralpha gene in non-hematopoietic derived tissues is 100% homologous to the IL-2Ralpha gene in lymphocytes. Petitto and Huang showed that cDNA from the full length coding region of saline-perfused mouse forebrains exactly matched to the corresponding lymphocyte IL-2Ralpha cDNA sequence (77). Others reported that the mRNA transcripts were of different lengths but the differences in the size of the mRNA transcripts are from the untranslated regions (77).

A study of 93 patients with esophageal squamous cell carcinoma (ESCC) not only examined the sIL-2Ralpha blood levels, but also performed immunohistochemistry (IHC) and *in situ* hybridization (ISH) on the excised esophageal tissue and compared to normal tissues (37). It was found that patients with sIL-2Ralpha levels > 1500 pg/ml had a poorer prognosis than those with levels < 1500 pg/ml. Additionally, the sIL-2Ralpha levels correlated well with Tumor-Node-Metastasis Classification, tumor stage, and reading score from IHC (37). Furthermore, this study showed that in 28 of the 93 patients (30%), IL-2Ralpha mRNA was identified by ISH analysis (37). These data indicate that lymphocytes are not the only source of IL-2Ralpha in tumors and that further study into the expression of IL-2Ralpha in cancer cells is warranted.

sIL-2Ralpha levels are also a good prognostic indicator of lymph node metastasis in patients with gastric cancer (80). A total of 40 preoperative patients with gastric cancer were evaluated for the serum sIL-2Ralpha levels and correlated with disease stage, gross appearance, histopathologic grade, and immunosuppressive acidic protein (IAP). The results from this study revealed that sIL-2Ralpha levels in patients with gastric cancer were significantly higher than the normal controls (80). Although no correlation was found when examining the histopathologic grade, there were significantly higher levels of IL-2Ralpha in patients with lymph node metastasis and IAP positive patients than those without metastasis and IAP expression (80).

Head-and-neck cancer is currently staged clinically based on size, lymph node involvement, and metastasis. However, these staging parameters do not provide reliable information regarding recurrence or future metastasis (81, 82). A multivariate prospective study involving 234 head-and-neck squamous cell carcinoma cancer (HNSCC) patients was performed by collecting plasma at the time of diagnosis and analyzing their sIL-2Ralpha levels (83). High serum soluble levels of IL-2Ralpha corresponded with a shorter survival time than those with lower levels. Additionally, it was found that distant-metastasis was also correlated with sIL-2Ralpha (83).

3.3. Tissue culture models

3.3.1. IL-2Ralpha transfectants

In order to study the relationship between IL-2R expression and cancer, stable transfectants of IL-2Ralpha/gamma and vector control (LacZ) were made with a squamous cell carcinoma of the head-and-neck cell line (PCI-13) (84, 85). Using plasmid vectors containing the IL-2R alpha chain gene under the control of a cytomegalovirus promoter or the IL-2R gamma chain gene under the control of a Rous sarcoma virus promoter, the IL-2R genes were transferred by lipofection into HNSCC cell lines. Stable transfectants were selected, cloned by limiting dilution, and clones were compared with the parental cell lines and vector control cells. The *IL-2Ralpha* transfectants expressed IL-2Ralpha on the cell surface, as measured by flow cytometry, in contrast to the parental or LacZ gene-transfected control cells, which expressed no detectable IL-2Ralpha (85). *IL-2Rgamma* transfectants showed increased levels of mRNA for IL-2Rgamma and also stained with anti-gamma chain Abs (85). Both the transfected and parental tumor cell lines expressed comparable levels of IL-2Rbeta on the cell surface (35).

3.3.2. Molecular characteristics

The genetically engineered cells, as described above, were examined for molecular and phenotypic changes. We found that the overexpression of IL-2Ralpha in PCI-13 cells is essential for anchorage-independent cellular growth (67). The enhanced transforming activity in IL-2Ralpha+ cells was accompanied by increased proliferation rates.

We then examined whether IL-2Ralpha expression could accelerate cell cycle progression. Indeed, IL-2Ralpha+ cells have an accelerated doubling time, decreased G_1 population, and increased G_2/M population (67). It is also possible that IL-2Ralpha+ cells have altered mitotic checkpoints or perhaps contain some tetraploid population (86). If so, IL-2Ralpha+ cells might acquire a chromosomal instability, which may contribute to the enhanced tumor aggressiveness.

To study the molecular players that are affected by IL-2Ralpha during the cell cycle, expression of several cyclins and cdks as well as proliferating cell nuclear antigen was determined. We found that levels of cyclin D1 and cdk4 proteins were increased in IL-2Ralpha+ cells, which should be responsible for the accelerated growth rate (87, 88). In addition, IL-2Ralpha+ cells have increased levels of cyclin A and cdk2 proteins, which might contribute to their increased proliferative activity and/or to the acquisition of anchorage-independent cellular growth (89-91). No change was observed in proliferating cell nuclear antigen. Other studies indicate that very high expression of IL-2Ralpha in spontaneously immortalized IL-2-dependent T lymphoblasts display enhanced

expression of cyclin D2, cdk4, cyclin E and/or c-myc (92, 93). These data indicate that IL-2Ralpha overexpression triggers signal transduction pathways driving the cancer cell cycle progression.

One mechanism for tumor cell accumulation is inhibition of apoptosis (94, 95), highly regulated cellular suicide program (96). Apoptosis regulation involves a balance of proapoptotic (e.g., Bad, Bax, Bid) and anti-apoptotic (e.g., Bcl-2, Bcl-X_L) molecules. The caspases, a family of cysteine proteases, are highly conserved and carry out step-by-step proteolysis of critical cellular proteins, leading to subsequent cellular apoptosis. IL-2Ralpha+ cells evade apoptosis induced by three apoptosis-inducing drugs, ALLN, VP-16, and taxol (67). High IL-2Ralpha expression leads to increased protein expression of Bcl-X_L or Bcl-2, both of which inhibit apoptosis (94, 95, 97). It has been shown that mitochondrial localization of Bcl-X_L and Bcl-2 is required for their anti-apoptotic functions (98). We determined that Bcl-X_L protein was present in both the mitochondria and cytosol of IL-2Ralpha overexpressing cells, while Bcl-2 is found solely in the mitochondria (67). These data suggest that high levels of mitochondrial Bcl-X_L and Bcl-2 proteins might contribute to the observed drug resistance in IL-2Ralpha+ cells.

Previously, by using the IL-2 specific antisense oligonucleotide to block synthesis of endogenous IL-2 in PCI-13 tumor cells, we also observed increased levels of p27 as well as p21 (99). The antisense oligonucleotides specific for p27 or p21 blocked expression of these proteins but not of IL-2 (99). Thus, endogenous IL-2 is important in regulating expression of p27 as well as p21 and, therefore, in controlling cell cycle progression of tumor cells, while its own expression remains independent of the CDK inhibitors (99).

In summary, IL-2Ralpha-overexpressing PCI-13 cells showed an enhanced transforming activity, associated with increased proliferation rates and drug resistance. These data are consistent with the clinical observation that many tumors and leukemic neoplasms with overexpression of IL-2Ralpha have a progressive phenotype and correlate with a poor prognosis for the patient (34-36, 41, 51, 66).

4. MONOCLONAL ANTIBODIES AGAINST IL-2RALPHA

Initially the development of antibodies against IL-2R and IL-2 stimulation focused on inhibiting activated T-cells in patients with organ transplants to suppress the immune response and prevent organ rejection. There are currently at least three monoclonal antibodies, which are discussed below, that provide promising new data for the treatment of hematopoietic derived cancers and possibly treatment of solid tumors (50, 100, 101).

4.1. 7G7/B6

7G7/B6 is a monoclonal antibody (mAb) against IL-2Ralpha developed in 1985. 7G7/B6 was derived from murine splenocytes immune to influenza virus-activated human T-cells and were fused with SP2/0 cells. This antibody has been found to bind IL-2Ralpha on an epitope that is different from the IL-2 binding site and distinctly different from the epitope bound by the mAb anti-Tac (100, 102). 7G7/B6 is capable of delaying the progression of leukemia in non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice infused with ATL cells (103). This study is particularly interesting because 7G7/B6 does not interfere with the binding of IL-2 so the mechanism of action must be something other than cytokine deprivation (103). The authors postulated that an antibody-dependent cellular cytotoxicity mediated by FcR-expressing cells may occur or 7G7/B6 directly induces apoptosis in the ATL cells (103).

4.2. Murine anti-Tac (MAT)

In 1981 a murine mAb against IL-2Ralpha (Tac) was derived using the somatic cell fusion technique (50). Briefly, a BALB/c mouse was immunized with cultured T cells from a patient with activated T cells due to mycosis fungoides. The spleen cells were then removed and fused with NS-1 mouse myeloma cells, cultured for selective IL-2R expression, and then inserted into a BALB/c mouse for antibody production (50). MAT has been shown to successfully inhibit ATL growth and prolong the survival of tumor bearing mice (103). Additionally, a therapeutic trial with MAT in 19 patients with ATL showed that MAT successfully lead to a partial remission in 4 patients and a complete remission in 2 patients lasting from 9 weeks up to 3 years (104). In general, the patients responded well to the intravenous administration of MAT and did not have any modification to the normal blood cellular makeup (18 of 19) (104).

4.3. Humanized anti-Tac (HAT; daclizumab; Zenepax)

Although MAT antibody was tolerated well in patients, the usefulness of this antibody treatment in patients is limited due to the short half-life in humans (40 h) and the development of human anti-murine antibodies (101, 104). To combat this issue, a humanized form of MAT (HAT; daclizumab) was developed by Waldmann's group of the National Cancer Institute. The antibody retained all the complementary determining regions from the mouse, but the Fc region was human IgG1 (105). The pharmacokinetics with daclizumab is much improved with a half-life that is extended to 20 days (101). The therapeutic focus with daclizumab has been mainly involved in suppressing allograft rejection (primarily renal) and T cell autoimmune disorders (106, 107). However, the recent use of daclizumab in conjunction with radionucliotides, small molecule inhibitors, and toxins has been evaluated for the treatment of a variety of lymphoid neoplasms (108-111).

In a murine model of adult T-cell leukemia, daclizumab proved very effective. An *in vivo* model of ATL was developed in NOD/SCID mice by injecting ATL cells into the mice, followed by treatment with daclizumab (100 μg/mouse) after the serum levels of sIL-2Ralpha reached >1000pg/ml (103). Daclizumab induced complete remissions in 4 of the 19 mice and partial remissions in the remaining mice, indicating that mAb treatment of hematopoietic cancers with IL-2Ralpha expression may be

a viable chemotherapeutic treatment in the near future (103).

5. POTENTIAL USE OF DACLIZUMAB AS AN ANTICANCER DRUG

5.1. Effects on proliferation and cell cycle

To further elucidate the role of IL-2Ralpha overexpression in tumor growth and survival, we assessed the effect that daclizumab has on the proliferative potential and cell cycle in HNSCC cells stably transfected with *IL-2Ralpha* and vector control (LacZ) (85). Although daclizumab is currently approved for treatment in renal transplant patients to abrogate IL-2-mediated activation of lymphocytes, a critical pathway in the cellular immune response involved in allograft rejection (107), the detailed molecular mechanisms are unclear. We hypothesized that daclizumab treatment in IL-2Ralpha+ cells would lead to inhibition of the downstream proliferative signaling pathways mediated by IL-2Ralpha expression.

We found that daclizumab effectively inhibited the proliferation of IL-2Ralpha+ cells, but not the vector control cells (112). The cell cycle distribution pattern differs dramatically between the untreated IL-2Ralpha+ and LacZ cells: while 59% of the IL-2Ralpha+ cells are in G_2-M phase, 50% of the LacZ cells reside in G_0-G_1 phase (67, 112). Treatment with daclizumab for 72 h effectively inhibited cell cycle progression in the IL-2Ralpha+ cells with a 57% increase of the cells in G_0-G_1, compared to LacZ, which only increased ~20%. A decrease in cyclin A protein expression (dose- and time-dependent) was observed only in IL-2Ralpha+ cells (112). Therefore, daclizumab selectively and effectively inhibits cell cycle progression in IL-2Ralpha+ cells.

5.2. Effects on regulators of apoptosis

Although the importance of sIL-2Ralpha shed from the surface of infiltrating lymphatic cells at solid tumor sites is well documented, the role IL-2Ralpha plays in cell survival of solid tumorigenic cells is not well characterized. If the cell surface receptor is responsible for the anti-apoptotic properties observed in both a cell culture model and in excised tumor samples, then inhibition of IL-2Ralpha should lead to apoptosis (36, 67). Although the vector control cells express very low levels of endogenous IL-2Ralpha on their cell membrane, they should not be dependent upon IL-2Ralpha signaling for cell survival. In fact, the vector cells have vastly different cell cycle and anti-apoptotic protein expression phenotypes compared to IL-2Ralpha+ cells, indicating that *IL-2Ralpha* transfectants have become dependent upon IL-2Ralpha-mediated signaling pathways. Daclizumab did not induce caspase-3 activation in LacZ cells. Conversely, IL-2Ralpha+ cells treated with daclizumab induced caspase-3 activity (3.5-fold) and increased the overall caspase activity by ~50% (112).

The mechanism of drug resistance in tumors overexpressing IL-2Ralpha is not entirely understood. Previously we reported that IL-2Ralpha+ cells are resistant to a tripeptidyl proteasome inhibitor (LLnL) and two chemotherapeutic drugs (VP-16 and taxol) (67). Transcription of anti-apoptotic genes, such as Bcl-2 and Bcl-X_L through the Jak-Stat pathway, driven by IL-2Ralpha expression should be inhibited by daclizumab, leading to increased sensitivity to apoptosis inducing drugs. To test this hypothesis, we pretreated cells with daclizumab, followed by introduction of LLnL, VP-16, and Taxol. We found that IL-2Ralpha+ cells were sensitized by the mAb, but the effect was moderate (112). A possible explanation for the modest effect of daclizumab on the sensitization to the apoptosis-inducing drugs may be linked to the arrest of the cells in G_0/G_1 after daclizumab treatment (112). Previous reports have shown that arresting cells in G_0/G_1 confers resistance to multiple apoptotic stimuli (113-115). Most chemotherapeutic drugs target actively cycling cells. For instance, VP-16 targets topoisomerase II, which is responsible for cleavage and rejoining of double-stranded DNA, allowing the separation of intertwined DNA strands essential for DNA replication and transcription (116). Taxol binds to tubulin, stabilizing their polymerization and leading to inhibition of chromosome separation in anaphase (117). Thus, resting cells do not provide an effective target for the drugs.

In addition, daclizumab may not provide an additive or synergistic effect to cell death-inducing drugs (LLnL, VP-16, and taxol), which is also probably due to the inability to decrease the anti-apoptotic protein Bcl-X_L, which is overexpressed in IL-2Ralpha cells *versus* control cells (67, 112). While a dose- and time-dependent decrease in Bcl-2 protein expression was observed after 25 μg/ml treatment with daclizumab in IL-2Rlpha+ cells, no change in Bcl-X_L expression was observed (112).

5.3. mAbs combined with other chemotherapeutics

5.3.1. Toxins

The combination of anti-Tac fused to immunotoxins show much promise for the treatment of cancers with high expression of IL-2Ralpha (118-122). This type of combinatorial molecule was first developed at the National Cancer Institute (123). Briefly, a truncated immunotoxin (Pseudomonas exotoxin) was fused to DNA elements encoding the variable region of the anti-Tac monoclonal antibody to produce anti-Tac(Fv)-PE40, which was amplified in Escherichia coli. Anti-Tac(Fv)-PE40 had highly selective cytotoxic activity against cells expressing IL-2Ralpha, but not to receptor-negative cells (123). Similarly, a combinatorial anti-Tac(Fv) fused to diphtherial toxin, DT388-anti-Tac(Fv), also had selective activity against IL-2Ralpha+ cells and was also able to kill proliferating T-cells in a mixed leukocyte reaction (124). Evaluation of anti-Tac(Fv)-PE40 in mice revealed that the fused antibody could be detected rapidly in the blood after intraperitoneal injection (125).

Phase I and II clinical trials indicate that DAB389-IL-2, a diphtheria linked toxin, is most active against cutaneous T cell lymphoma (CTCL). Five complete responses and seven partial responses were observed out of the 35 patients assessed (118). Most side effects consisting of nausea, vomiting, hypotension, fever/chills and elevated liver enzymes were mild and transient (118). In another study, malignant cells from a hairy cell leukemia (HCL)

patient were removed and assessed for their responsiveness to anti-Tac(Fv)-PE38, pseudomonas exotoxin linked anti-Tac(Fv) (126). The malignant cells were extremely sensitive to the cytotoxic effect of anti-Tac(Fv)-PE38 with an IC50 of 1.1 ng/ml. The patient was then given anti-Tac(Fv)-PE38 intravenously and showed a clinical response (126). Based on this data, Robbins et al., obtained leukemic cells from 9 additional patients with (HCL) and found that all were sensitive to anti-Tac(Fv)-PE38 (IC50 0.5-6.0 ng/ml) (126).

A phase I trial using anti-Tac(Fv)-PE38 in 35 patients with various hematologic malignancies showed potent clinical activity and relatively nonimmunogenicity (122). One HCL patient achieved a complete remission and seven partial responses were observed in patients with cutaneous T-cell lymphoma, hairy cell leukemia, Hodgkin's disease, chronic lymphocytic leukemia, and adult T-cell leukemia. All the responding patients had log reductions in their malignant cell population (122).

5.3.2. Radiotherapy

The conjugation of anti-Tac to radioisotopes has shown promise in inhibiting tumor cell growth with IL-2Ralpha expression. An *in vitro* study to determine the effectiveness of Bismuth 212 (^{212}Bi; alpha-particle emitting radionuclide) coupled to anti-Tac in a mixed lymphocyte reaction showed that cytotoxic T cells were eliminated and that ^{212}Bi-anti-Tac selectively inhibited IL-2Ralpha expression cells (127). The activity of 212Bi-anti-Tac was successfully inhibited with the addition of excess anti-Tac and the therapeutic reactions were most effective when added just prior to the peak of alloproliferative response, when IL-2Ralpha receptor expression is at the highest (127).

In an animal study, SP2 murine plasmacytoma cells were transfected with IL-2Ralpha to produce SP2/Tac cell line. These cells were then injected either i.p. or s.c. into nude mice, followed by administration of anti-Hat conjugated to 212Bi (108). 212Bi-anti-Hat was particularly effective, in a dose-dependent manner, when administered by i.p., 3 days following i.p. inoculation of tumor cells, and lead to a prolongation of tumor-free survival (75%) (108). However, the administration of 212Bi-anti-Hat by i.v. 3 days after inoculation of tumor cells by s.c. lead to a significant decrease in the prevention of tumor occurrence (30%). Unfortunately, 212Bi-anti-Hat was not effective at reducing large, established s.c. SP2/Tac tumors when administered i.v. at larger doses (200 microCi/animal) (108).

In a phase I and II clinical trail, 18 patients with ATL were given 5- to 15-mCi of anti-Tac conjugated to Yttrium 90 (90Y), a beta-emitting radionuclide. Phase I (9 patients) consisted of a dose-escalating trial and phase II (9 patients) was based on a uniform dosage of 10 mCi (128). A total of 9 responses were observed with 7 partial and 2 complete remissions. These data indicate that 90Y-anti-Tac therapy may provide a useful treatment of this extremely aggressive cancer (128).

5.3.3. Small molecule drugs

PS-341 (Velcade) is a dipeptidyl boronic acid analog with selective activity against the 20S proteasome (129). The proteasome is not only responsible for degradation of obsolete and mis-folded proteins, but it plays a major role in regulating both cell cycle and apoptosis (130-134). For instance, nuclear factor kappa B (NFκB) transactivates numerous proteins that sustain proliferation and suppress apoptosis (135) and its inhibitor, inhibitor of NFκB (IκB), is degraded by the proteasome (136). Therefore, inhibiting the proteolytic activity of the proteasome will lead to accumulation of IκB and subsequent apoptosis (135). An *in vivo* study in nude mice with injections of MET-1 cells (ATL) reports that administration of PS-341 conjugated to anti-Hat lead to a significant number of complete remissions in the mice (109). Conversely, anti-Hat or PS-341 treatment alone was not as efficacious, leading to only partial remissions (anti-Hat) or no response at all (PS-341).

Most recently, Waldmann's group at the National Cancer Institute, performed a study using flavopiridol in combination with anti-Hat (110). Flavopiridol is a small molecule inhibitor of the cyclin dependent kinases (cdks), which coordinate and control the progression of cellular replication (137, 138). A murine model of ATL was developed and treatments with flavopiridol alone (2.5 mg/kg for 5 days), anti-Hat alone (100 ? g weekly for 4 weeks), or the combination of both regimens were evaluated (110). The median survival of flavopiridol and anti-Hat alone were 51 and 98 days respectively, compared to the mean 144 days of the combination treated group (110).

6. CONCLUSIONS AND FUTURE DIRECTIONS

The role of IL-2Ralpha in human cancers, especially solid tumors, is gradually becoming better defined. Initially, many studies assumed that amplified levels of sIL-2Ralpha found in patients with cancer were due to infiltrating lymphocytes, however, the tumors themselves may be shedding IL-2Ralpha. IL-2Ralpha expression leads to increased proliferation, anti-apoptotic protein expression, and drug resistance, as shown in human head-and-neck cancer cell lines. The development of monoclonal antibodies against IL-2Ralpha is a significant advancement for patients with cancers that overexpress IL-2Ralpha, particularly those with hematopoietic malignancies. The combination of small molecule inhibitors and immunotoxins with anti-Tac antibodies increases treatment efficacy with little side effects to the patients. However, using anti-Tac in patients with solid tumors expressing IL-2Ralpha may be problematic due to inadequate delivery of the mAb directly to the tumor site. In conclusion, IL-2Ralpha may play a significant role in tumorigenesis and resistance to chemotherpeutic drugs. Therefore, continued characterization of IL-2Ralpha's function in cancer is warranted.

7. ACKNOWLEDGMENTS

We thank Dr. Jan Konrad Siwicki and Ms. Kristin Landis-Piwowar for their critical reading of the manuscript and Barbara Ann Karmanos Cancer Institute for research support to this project (to Q. P. D.).

8. REFERENCES

1. Morgan, D. A., F. W. Ruscetti & R. Gallo: Selective in vitro growth of T lymphocytes from normal human bone marrows. *Science* 193, 1007-1008 (1976)
2. Gillis, S., N. A. Union, P. E. Baker & K. A. Smith: The in vitro generation and sustained culture of nude mouse cytolytic T-lymphocytes. *J Exp Med* 149, 1460-1476 (1979)
3. Robb, R. J., W. C. Greene & C. M. Rusk: Low and high affinity cellular receptors for interleukin 2. Implications for the level of Tac antigen. *J Exp Med* 160, 1126-1146 (1984)
4. Thornton, A. M., C. A. Piccirillo & E. M. Shevach: Activation requirements for the induction of CD4+CD25+ T cell suppressor function. *Eur J Immunol* 34, 366-376 (2004)
5. Whiteside, T. L. & R. B. Herberman: The role of natural killer cells in immune surveillance of cancer. *Curr Opin Immunol* 7, 704-710 (1995)
6. Sharon, M., R. D. Klausner, B. R. Cullen, R. Chizzonite & W. J. Leonard: Novel interleukin-2 receptor subunit detected by cross-linking under high-affinity conditions. *Science* 234, 859-863 (1986)
7. Takemoto, H.: Murine interleukin-2 receptor subunits differentially detected with anti-interleukin-2 monoclonal antibodies. *FEBS Lett* 250, 331-335 (1989)
8. Anderson, D. M., S. Kumaki, M. Ahdieh, J. Bertles, M. Tometsko, A. Loomis, J. Giri, N. G. Copeland, D. J. Gilbert & N. A. Jenkins: Functional characterization of the human interleukin-15 receptor alpha chain and close linkage of IL15RA and IL2RA genes. *J Biol Chem* 270, 29862-29869 (1995)
9. Kimura, Y., T. Takeshita, M. Kondo, N. Ishii, M. Nakamura, J. Van Snick & K. Sugamura: Sharing of the IL-2 receptor gamma chain with the functional IL-9 receptor complex. *Int Immunol* 7, 115-120 (1995)
10. Russell, S. M., A. D. Keegan, N. Harada, Y. Nakamura, M. Noguchi, P. Leland, M. C. Friedmann, A. Miyajima, R. K. Puri & W. E. Paul: Interleukin-2 receptor gamma chain: a functional component of the interleukin-4 receptor. *Science* 262, 1880-1883 (1993)
11. Noguchi, M., Y. Nakamura, S. M. Russell, S. F. Ziegler, M. Tsang, X. Cao & W. J. Leonard: Interleukin-2 receptor gamma chain: a functional component of the interleukin-7 receptor. *Science* 262, 1877-1880 (1993)
12. Taniguchi, T. & Y. Minami: The IL-2/IL-2 receptor system: a current overview. *Cell* 73, 5-8 (1993)
13. Leonard, W. J., J. M. Depper, T. Uchiyama, K. A. Smith, T. A. Waldmann & W. C. Greene: A monoclonal antibody that appears to recognize the receptor for human T-cell growth factor; partial characterization of the receptor. *Nature* 300, 267-269 (1982)
14. Benedict, S. H., G. B. Mills & E. W. Gelfand: Interleukin 2 activates a receptor-associated protein kinase. *J Immunol* 139, 1694-1697 (1987)
15. Ellery, J. M. & P. J. Nicholls: Alternate signalling pathways from the interleukin-2 receptor. *Cytokine Growth Factor Rev* 13, 27-40 (2002)
16. Boussiotis, V. A., D. L. Barber, T. Nakarai, G. J. Freeman, J. G. Gribben, G. M. Bernstein, A. D. D'Andrea, J. Ritz & L. M. Nadler: Prevention of T cell anergy by signaling through the gamma c chain of the IL-2 receptor. *Science* 266, 1039-1042 (1994)
17. Waldmann, T. A., S. Dubois & Y. Tagaya: Contrasting roles of IL-2 and IL-15 in the life and death of lymphocytes: implications for immunotherapy. *Immunity* 14, 105-110 (2001)
18. Aaronson, D. S. & C. M. Horvath: A road map for those who don't know JAK-STAT. *Science* 296, 1653-1655 (2002)
19. Russell, S. M., J. A. Johnston, M. Noguchi, M. Kawamura, C. M. Bacon, M. Friedmann, M. Berg, D. W. McVicar, B. A. Witthuhn, O. Silvennoinen & et al.: Interaction of IL-2R beta and gamma c chains with Jak1 and Jak3: implications for XSCID and XCID. *Science* 266, 1042-1045 (1994)
20. Gonzalez-Garcia, A., I. Merida, A. C. Martinez & A. C. Carrera: Intermediate affinity interleukin-2 receptor mediates survival via a phosphatidylinositol 3-kinase-dependent pathway. *J Biol Chem* 272, 10220-10226 (1997)
21. Satoh, T., Y. Minami, T. Kono, K. Yamada, A. Kawahara, T. Taniguchi & Y. Kaziro: Interleukin 2-induced activation of Ras requires two domains of interleukin 2 receptor beta subunit, the essential region for growth stimulation and Lck-binding domain. *J Biol Chem* 267, 25423-25427 (1992)
22. Delespine-Carmagnat, M., G. Bouvier, G. Allee, R. Fagard & J. Bertoglio: Biochemical analysis of interleukin-2 receptor beta chain phosphorylation by p56(lck). *FEBS Lett* 447, 241-246 (1999)
23. Turner, B., U. Rapp, H. App, M. Greene, K. Dobashi & J. Reed: Interleukin 2 induces tyrosine phosphorylation and activation of p72-74 Raf-1 kinase in a T-cell line. *Proc Natl Acad Sci U S A* 88, 1227-1231 (1991)
24. Ravichandran, K. S., V. Igras, S. E. Shoelson, S. W. Fesik & S. J. Burakoff: Evidence for a role for the phosphotyrosine-binding domain of Shc in interleukin 2 signaling. *Proc Natl Acad Sci U S A* 93, 5275-5280 (1996)
25. Li, N., A. Batzer, R. Daly, V. Yajnik, E. Skolnik, P. Chardin, D. Bar-Sagi, B. Margolis & J. Schlessinger: Guanine-nucleotide-releasing factor hSos1 binds to Grb2 and links receptor tyrosine kinases to Ras signalling. *Nature* 363, 85-88 (1993)
26. Miyazaki, T., Z. J. Liu, A. Kawahara, Y. Minami, K. Yamada, Y. Tsujimoto, E. L. Barsoumian, R. M. Permutter & T. Taniguchi: Three distinct IL-2 signaling pathways mediated by bcl-2, c-myc, and lck cooperate in hematopoietic cell proliferation. *Cell* 81, 223-231 (1995)
27. Kawahara, A., Y. Minami, T. Miyazaki, J. N. Ihle & T. Taniguchi: Critical role of the interleukin 2 (IL-2) receptor gamma-chain-associated Jak3 in the IL-2-induced c-fos and c-myc, but not bcl-2, gene induction. *Proc Natl Acad Sci U S A* 92, 8724-8728 (1995)
28. Reinecker, H. .C & D. K. Podolsky: Human intestinal epithelial cells express functional cytokine receptors sharing the common gamma c chain of the interleukin 2 receptor. *Proc Natl Acad Sci U S A* 92, 8353-8357 (1995).
29. Sadlack, B., J. Lohler, H. Schorle, G. Klebb, H. Haber, E. Sickel, R. J. Noelle & I. Horak: Generalized autoimmune disease in interleukin-2-deficient mice is triggered by an uncontrolled activation and proliferation of CD4+ T cells. *Eur J Immunol* 25, 3053-3059 (1995)

30. Schorle, H., T. Holtschke, T. Hunig, A. Schimpl & I. Horak: Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. *Nature* 352, 621-624 (1991)
31. Sharfe, N., H. K. Dadi, M. Shahar & C. M. Roifman: Human immune disorder arising from mutation of the alpha chain of the interleukin-2 receptor. *Proc Natl Acad Sci U S A* 94, 3168-3171 (1997)
32. Balasubramanian, S., T. Chernov-Rogan, A. M. Davis, E. Whitehorn, E. Tate, M. P. Bell, G. Zurawski & R. W. Barrett: Ligand binding kinetics of IL-2 and IL-15 to heteromers formed by extracellular domains of the three IL-2 receptor subunits. *Int Immunol* 7, 1839-1849 (1995)
33. Vamosi, G., A. Bodnar, G. Vereb, A. Jenei, C. K. Goldman, J. Langowski, K. Toth, L. Matyus, J. Szollosi, T. A. Waldmann & S. Damjanovich: IL-2 and IL-15 receptor alpha-subunits are coexpressed in a supramolecular receptor cluster in lipid rafts of T cells. *Proc Natl Acad Sci U S A* 101, 11082-11087 (2004)
34. McDoniels-Silvers, A. L., G. D. Stoner, R. A. Lubet & M. You: Differential expression of critical cellular genes in human lung adenocarcinomas and squamous cell carcinomas in comparison to normal lung tissues. *Neoplasia* 4, 141-150 (2002)
35. Rimoldi, D., S. Salvi, F. Hartmann, M. Schreyer, S. Blum, L. Zografos, S. Plaisance, B. Azzarone & S. Carrel: Expression of IL-2 receptors in human melanoma cells. *Anticancer Res* 13, 555-564 (1993)
36. Royuela, M., M. P. De Miguel, F. R. Bethencourt, B. Fraile, M. I. Arenas & R. Paniagua: IL-2, its receptors, and bcl-2 and bax genes in normal, hyperplastic and carcinomatous human prostates: immunohistochemical comparative analysis. *Growth Factors* 18, 135-146 (2000)
37. Wang, L. S., K. C. Chow, W. Y. Li, C. C. Liu, Y. C. Wu & M. H. Huang: Clinical significance of serum soluble interleukin 2 receptor-alpha in esophageal squamous cell carcinoma. *Clin Cancer Res* 6, 1445-1451 (2000)
38. Araki, K., K. Harada, K. Nakamoto, M. Shiroma & T. Miyakuni: Clinical significance of serum soluble IL-2R levels in patients with adult T cell leukaemia (ATL) and HTLV-1 carriers. *Clin Exp Immunol* 119, 259-263 (2000)
39. Pui, C. H., S. H. Ip, S. Iflah, F. G. Behm, B. H. Grose, R. K. Dodge, W. M. Crist, W. L. Furman, S. B. Murphy & G. K. Rivera: Serum interleukin 2 receptor levels in childhood acute lymphoblastic leukemia. *Blood* 71, 1135-1137 (1988)
40. Knauf, W. U., I. Langenmayer, B. Ehlers, B. Mohr, D. Adorf, C. H. Nerl, M. Hallek, T. H. Zwingers, B. Emmerich & E. Thiel: Serum levels of soluble CD23, but not soluble CD25, predict disease progression in early stage B-cell chronic lymphocytic leukemia. *Leuk Lymphoma* 27, 523-532 (1997)
41. Semenzato, G., R. Foa, C. Agostini, R. Zambello, L. Trentin, F. Vinante, F. Benedetti, M. Chilosi & G. Pizzolo: High serum levels of soluble interleukin 2 receptor in patients with B chronic lymphocytic leukemia. *Blood* 70, 396-400 (1987)
42. Tsai, M. H., S. H. Chiou & K. C. Chow: Effect of platelet activating factor and butyrate on the expression of interleukin-2 receptor alpha in nasopharyngeal carcinoma cells. *Int J Oncol* 19, 1049-1055 (2001)
43. Barton, D. P., D. K. Blanchard, A. F. Wells, S. V. Nicosia, W. S. Roberts, D. Cavanagh & J. Y. Djeu: Expression of interleukin-2 receptor alpha (IL-2R alpha) mRNA and protein in advanced epithelial ovarian cancer. *Anticancer Res* 14, 761-772 (1994)
44. Berghella, A. M., I. Contasta, P. Pellegrini, T. Del Beato & D. Adorno: Peripheral blood immunological parameters for use as markers of pre-invasive to invasive colorectal cancer. *Cancer Biother Radiopharm* 17, 43-50 (2002)
45. Murakami, S., A. Satomi, K. Ishida, H. Murai & Y. Okamura: Serum soluble interleukin-2 receptor in colorectal cancer. *Acta Oncol* 33, 19-21 (1994)
46. Murakami, S., R. Hirayama, A. Satomi, K. Okubo, M. Matsuki, H. Sakata & Y. Tsuji: Serum Soluble Interleukin-2 Receptor Levels in Patients with Breast Cancer. *Breast Cancer* 4, 25-28 (1997)
47. Rubin, L. A. & D. L. Nelson: The soluble interleukin-2 receptor: biology, function, and clinical application. *Ann Intern Med* 113, 619-627 (1990)
48. Yoshida, M., I. Miyoshi & Y. Hinuma: A retrovirus from human leukemia cell lines: its isolation, characterization, and implication in human adult T-cell leukemia (ATL). *Princess Takamatsu Symp* 12, 285-294 (1982)
49. Yoshida, M., I. Miyoshi & Y. Hinuma: Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. *Proc Natl Acad Sci U S A* 79, 2031-2035 (1982)
50. Uchiyama, T., S. Broder & T. A. Waldmann: A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. I. Production of anti-Tac monoclonal antibody and distribution of Tac (+) cells. *J Immunol* 126, 1393-1397 (1981)
51. Waldmann, T. A., W. J. Leonard, J. M. Depper, M. Kronke, C. K. Goldman, T. Oh & W. C. Greene: Interleukin-2 receptor expression in retrovirus associated adult T-cell leukemia. *Princess Takamatsu Symp* 15, 259-268 (1984)
52. Uchiyama, T., T. Hori, M. Tsudo, Y. Wano, H. Umadome, S. Tamori, J. Yodoi, M. Maeda, H. Sawami & H. Uchino: Interleukin-2 receptor (Tac antigen) expressed on adult T cell leukemia cells. *J Clin Invest* 76, 446-453 (1985)
53. Guipaud, O., L. Deriano, H. Salin, L. Vallat, L. Sabatier, H. Merle-Beral & J. Delic: B-cell chronic lymphocytic leukaemia: a polymorphic family unified by genomic features. *Lancet Oncol* 4, 505-514 (2003)
54. Tsilivakos, V., A. Tsapis, S. Kakolyris, P. Iliakis, M. Perraki & V. Georgoulias: Characterization of interleukin 2 receptors on B-cell chronic lymphocytic leukemia cells. *Leukemia* 8, 1571-1578 (1994)
55. Yagura, H., T. Tamaki, T. Furitsu, Y. Tomiyama, T. Nishiura, N. Tominaga, S. Katagiri, T. Yonezawa & S. Tarui: Demonstration of high-affinity interleukin-2 receptors on B-chronic lymphocytic leukemia cells: functional and structural characterization. *Blut*. 60, 181-186 (1990)
56. Bouroncle, B. A., B. K. Wiseman & C. A. Doan: Leukemic reticuloendotheliosis. *Blood* 13, 609-630 (1958)

57. Ambrosetti, A., G. Semenzato, M. Prior, M. Chilosi, F. Vinante, C. Vincenzi, R. Zanotti, L. Trentin, A. Portuese, F. Menestrina & et al.: Serum levels of soluble interleukin-2 receptor in hairy cell leukaemia: a reliable marker of neoplastic bulk. *Br J Haematol* 73, 181-186 (1989)
58. Richards, J. M., R. Mick, J. M. Latta, K. Daly, M. J. Ratain, J. W. Vardiman & H. M. Golomb: Serum soluble interleukin-2 receptor is associated with clinical and pathologic disease status in hairy cell leukemia. *Blood* 76, 1941-1945 (1990)
59. Chrobak, L., K. Podzimek, L. Pliskova, Z. Kerekes, P. Zak, J. Voglova, J. Spacek & V. Palicka: Serum soluble IL-2 receptor as a reliable and noninvasive marker of disease activity in patients with hairy cell leukemia. *Neoplasma* 43, 321-325 (1996)
60. Thomas, D. A., S. O'Brien, C. Bueso-Ramos, S. Faderl, M. J. Keating, F. J. Giles, J. Cortes & H. M. Kantarjian: Rituximab in relapsed or refractory hairy cell leukemia. *Blood* 102, 3906-3911 (2003)
61. Nakase, K., K. Kita, A. Otsuji, H. Anazawa, S. Shirakawa, K. Nasu, H. Dohy, H. Tsutani & I. Tanaka: Interleukin-2 receptor alpha chain on acute myelocytic leukemia cells is involved in cell-to-cell interactions. *Leuk Res* 17, 17-21 (1993)
62. Weidmann, E., J. Brieger, L. Bergmann, D. Hoelzer & P. S. Mitrou: AML blasts variably express interleukin 2 receptor alpha, beta or gamma chains without measurable effects on proliferation, cytokine message expression or surface expression of adhesion molecules upon stimulation with interleukin 2. *Leuk Res* 19, 469-476 (1995)
63. Schumann, R. R., T. Nakarai, H. J. Gruss, M. A. Brach, U. von Arnim, C. Kirschning, L. Karawajew, W. D. Ludwig, J. C. Renauld, J. Ritz & F. Herrmann: Transcript synthesis and surface expression of the interleukin-2 receptor (alpha-, beta-, and gamma-chain) by normal and malignant myeloid cells. *Blood* 87, 2419-2427 (1996)
64. Nakase, K., K. Kita, H. Anazawa, K. Hoshino, S. Shirakawa, I. Tanaka & M. Tsudo: Induction of interleukin-2 receptor alpha chain expression of immature acute myelocytic leukemia cells. *Leuk Res* 18, 269-274 (1994)
65. Pizzolo, G., A. Rigo, R. Zanotti, F. Vinante, C. Vincenzi, M. Cassatella, G. Carra, G. Castaman, M. Chilosi, G. Semenzato, et al.: Alpha (p55) and beta (p75) chains of the interleukin-2 receptor are expressed by AML blasts. *Leukemia* 7, 418-425 (1993)
66. Garcia-Tunnon, I., M. Ricote, A. Ruiz, B. Fraile, R. Paniagua & M. Royuela: Interleukin-2 and its receptor complex (alpha, beta and gamma chains) in in situ and infiltrative human breast cancer: an immunohistochemical comparative study. *Breast Cancer Res* 6, R1-7 (2004)
67. Kuhn, D. J., D. M. Smith, S. Pross, T. L. Whiteside & Q. P. Dou: Overexpression of interleukin-2 receptor alpha in a human squamous cell carcinoma of the head and neck cell line is associated with increased proliferation, drug resistance, and transforming ability. *J Cell Biochem* 89, 824-836 (2003)
68. Ferdeghini, M., A. Gadducci, C. Prontera, G. Malagnino, A. Fanucchi, C. Annicchiarico, V. Facchini & R. Bianchi: Serum soluble interleukin-2 receptor assay in epithelial ovarian cancer. *Tumour Biol* 14, 303-309 (1993)
69. Vuoristo, M. S., S. Laine, H. Huhtala, L. M. Parvinen, M. Hahka-Kemppinen, M. Korpela, E. Kumpulainen & P. Kellokumpu-Lehtinen: Serum adhesion molecules and interleukin-2 receptor as markers of tumour load and prognosis in advanced cutaneous melanoma. *Eur J Cancer* 37, 1629-1634 (2001)
70. He, Y. G., E. Mayhew, J. Mellon & J. Y. Niederkorn: Expression and possible function of IL-2 and IL-15 receptors on human uveal melanoma cells. *Invest Ophthalmol Vis Sci*. 45, 4240-4246 (2004)
71. Garcia-Vazquez, M. D., M. D. Boyano, M. L. Canavate, J. Gardeazabal, A. G. de Galdeano, T. Lopez-Michelena, J. A. Raton, R. Izu, J. L. Diaz-Ramon & J. L. Diaz-Perez: Interleukin-2 enhances the growth of human melanoma cells derived form primary but not from metastatic tumours. *Eur Cytokine Netw*. 11, 654-661 (2000)
72. American Cancer Society. Cancer Facts and Figures. Atlanta, GA (2002) http://www.cancer.org/downloads/STT/CancerFacts&Figures2002TM.pdf
73. Linnoila, R. & S. Aisner: Pathology of Lung Cancer: an Exercise in Classification. In: Pathology of Lung Cancer: an Exercise in Classification. Eds: Johnson, B, Johnson, D, Wiley-Liss, New York, NY (1995)
74. Naumnik, W. & E. Chyczewska: The clinical significance of serum soluble interleukin 2 receptor (sIL-2R) concentration in lung cancer. *Folia Histochem Cytobiol* 39 Suppl 2, 185-186 (2001)
75. Ciacci, C., Y. R. Mahida, A. Dignass, M. Koizumi & D. K. Podolsky: Functional interleukin-2 receptors on intestinal epithelial cells. *J Clin Invest* 92, 527-532 (1993)
76. Plaisance, S., E. Rubinstein, A. Alileche, Y. Sahraoui, P. Krief, Y. Augery-Bourget, C. Jasmin, H. Suarez & B. Azzarone: Expression of the interleukin-2 receptor on human fibroblasts and its biological significance. *Int Immunol* 4, 739-746 (1992)
77. Petitto, J. M. & Z. Huang: Molecular cloning of the coding sequence of an interleukin-2 receptor alpha subunit cDNA in murine brain. *J Neuroimmunol* 59, 135-141 (1995)
78. Yasumura, S., W. C. Lin, E. Weidmann, P. Hebda & T. L. Whiteside: Expression of interleukin 2 receptors on human carcinoma cell lines and tumor growth inhibition by interleukin 2. *Int J Cancer* 59, 225-234 (1994)
79. Arzt, E., G. Stelzer, U. Renner, M. Lange, O. A. Muller & G. K. Stalla: Interleukin-2 and interleukin-2 receptor expression in human corticotrophic adenoma and murine pituitary cell cultures. *J Clin Invest* 90, 1944-1951 (1992)
80. Murakami, S., A. Satomi, K. Ishida, H. Murai, M. Matsuki & T. Hashimoto: Serum-soluble interleukin-2 receptor concentrations in patients with gastric cancer. *Cancer* 74, 2745-2748 (1994)
81. Smith, B. D., G. L. Smith, D. Carter, C. T. Sasaki & B. G. Haffty: Prognostic significance of vascular endothelial growth factor protein levels in oral and oropharyngeal squamous cell carcinoma. *J Clin Oncol* 18, 2046-2052 (2000)
82. Mineta, H., K. Miura, I. Suzuki, S. Takebayashi, H. Amano, K. Araki, H. Harada, K. Ichimura, J. P. Wennerberg & M. R. Dictor: Low p27 expression correlates with poor prognosis for patients with oral tongue squamous cell carcinoma. *Cancer* 85, 1011-1017 (1999)
83. Tartour, E., V. Mosseri, T. Jouffroy, L. Deneux, C. Jaulerry, F. Brunin, W. H. Fridman & J. Rodriguez: Serum

soluble interleukin-2 receptor concentrations as an independent prognostic marker in head and neck cancer. *Lancet* 357, 1263-1264 (2001)
84. Heo, D. S., C. Snyderman, S. M. Gollin, S. Pan, E. Walker, R. Deka, E. L. Barnes, J. T. Johnson, R. B. Herberman & T. L. Whiteside: Biology, cytogenetics, and sensitivity to immunological effector cells of new head and neck squamous cell carcinoma lines. *Cancer Res* 49, 5167-5175 (1989)
85. Lin, W. C., S. Yasumura & T. L. Whiteside: Transfer of interleukin 2 receptor genes into squamous cell carcinoma. Modification of tumor cell growth. *Arch Otolaryngol Head Neck Surg* 119, 1229-1235 (1993)
86. Khan, S. H. & G. M. Wahl: p53 and pRb prevent rereplication in response to microtubule inhibitors by mediating a reversible G1 arrest. *Cancer Research* 58, 396-401 (1998)
87. Imoto, M., Y. Doki, W. Jiang, E. K. Han & I. B. Weinstein: Effects of cyclin D1 overexpression on G1 progression-related events. *Exp Cell Res* 236, 173-180 (1997)
88. Meyerson, M., G. H. Enders, C. L. Wu, L. K. Su, C. Gorka, C. Nelson, E. Harlow & L. H. Tsai: A family of human cdc2-related protein kinases. *Embo J* 11, 2909-2917 (1992)
89. Yam, C. H., T. K. Fung & R. Y. Poon: Cyclin A in cell cycle control and cancer. *Cell Mol Life Sci* 59, 1317-1326 (2002)
90. Barrett, J. F., B. C. Lewis, A. T. Hoang, R. J. Alvarez Jr & C. V. Dang: Cyclin A links c-Myc to adhesion-independent cell proliferation. *J Biol Chem*. 270, 15923-5 (1995)
91. Guadagno, T. M., M. Ohtsubo, J. M. Roberts & R. K. Assoian: A link between cyclin A expression and adhesion-dependent cell cycle progression. *Science* 262, 1572-5 (1993)
92. Siwicki, J. K., Y. Hedberg, R. Nowak, M. Loden, J. Zhao, G. Landberg & G. Roos: Long-term cultured IL-2-dependent T cell lines demonstrate p16(INK4a) overexpression, normal pRb/p53, and upregulation of cyclins E or D2. *Exp Gerontol* 35, 375-388 (2000)
93. Siwicki, J. K., S. Degerman, K. H. Chrzanowska & G. Roos: Telomere maintenance and cell cycle regulation in spontaneously immortalized T-cell lines from Nijmegen breakage syndrome patients. *Exp Cell Res* 287, 178-189 (2003)
94. Reed, J. C.: Bcl-2 family proteins: regulators of apoptosis and chemoresistance in hematologic malignancies. *Semin Hematol* 34, 9-19 (1997)
95. Dalton, W. S. & R. Jove: Drug resistance in multiple myeloma: approaches to circumvention. *Semin Oncol* 26, 23-27 (1999)
96. Wyllie, A. H., J. F. Kerr & A. R. Currie: Cell death: the significance of apoptosis. *Int Rev Cytol* 68, 251-306 (1980)
97. Hengartner, M. O.: The biochemistry of apoptosis. *Nature* 407, 770-776 (2000)
98. Cory, S.: Regulation of lymphocyte survival by the bcl-2 gene family. *Annu Rev Immunol* 13, 513-543 (1995)
99. Reichert, T. E., S. Nagashima, Y. Kashii, J. Stanson, G. Gao, Q. P. Dou & T. L. Whiteside: Interleukin-2 expression in human carcinoma cell lines and its role in cell cycle progression. *Oncogene* 19, 514-525 (2000)
100. Rubin, L. A., C. C. Kurman, W. E. Biddison, N. D. Goldman & D. L. Nelson: A monoclonal antibody 7G7/B6, binds to an epitope on the human interleukin-2 (IL-2) receptor that is distinct from that recognized by IL-2 or anti-Tac. *Hybridoma* 4, 91-102 (1985)
101. Hakimi, J., R. Chizzonite, D. R. Luke, P. C. Familletti, P. Bailon, J. A. Kondas, R. S. Pilson, P. Lin, D. V. Weber, C. Spence & et al.: Reduced immunogenicity and improved pharmacokinetics of humanized anti-Tac in cynomolgus monkeys. *J Immunol* 147, 1352-1359 (1991)
102. Robb, R. J., C. M. Rusk & M. P. Neeper: Structure-function relationships for the interleukin 2 receptor: location of ligand and antibody binding sites on the Tac receptor chain by mutational analysis. *Proc Natl Acad Sci U S A* 85, 5654-5658 (1988)
103. Phillips, K. E., B. Herring, L. A. Wilson, M. S. Rickford, M. Zhang, C. K. Goldman, J. Y. Tso & T. A. Waldmann: IL-2Ralpha-Directed monoclonal antibodies provide effective therapy in a murine model of adult T-cell leukemia by a mechanism other than blockade of IL-2/IL-2Ralpha interaction. *Cancer Res* 60, 6977-6984 (2000)
104. Waldmann, T. A., J. D. White, C. K. Goldman, L. Top, A. Grant, R. Bamford, E. Roessler, I. D. Horak, S. Zaknoen, C. Kasten-Sportes & et al.: The interleukin-2 receptor: a target for monoclonal antibody treatment of human T-cell lymphotrophic virus I-induced adult T-cell leukemia. *Blood* 82, 1701-1712 (1993)
105. Queen, C., W. P. Schneider, H. E. Selick, P. W. Payne, N. F. Landolfi, J. F. Duncan, N. M. Avdalovic, M. Levitt, R. P. Junghans & T. A. Waldmann: A humanized antibody that binds to the interleukin 2 receptor. *Proc Natl Acad Sci U S A* 86, 10029-10033 (1989)
106. Nussenblatt, R. B., E. Fortin, R. Schiffman, L. Rizzo, J. Smith, P. Van Veldhuisen, P. Sran, A. Yaffe, C. K. Goldman, T. A. Waldmann & S. M. Whitcup: Treatment of noninfectious intermediate and posterior uveitis with the humanized anti-Tac mAb: a phase I/II clinical trial. *Proc Natl Acad Sci U S A* 96, 7462-7466 (1999)
107. Vincenti, F., R. Kirkman, S. Light, G. Bumgardner, M. Pescovitz, P. Halloran, J. Neylan, A. Wilkinson, H. Ekberg, R. Gaston, L. Backman & J. Burdick: Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. Daclizumab Triple Therapy Study Group. *N Engl J Med* 338, 161-165 (1998)
108. Hartmann, F., E. M. Horak, K. Garmestani, C. Wu, M. W. Brechbiel, R. W. Kozak, J. Tso, S. A. Kosteiny, O. A. Gansow, D. L. Nelson & et al.: Radioimmunotherapy of nude mice bearing a human interleukin 2 receptor alpha-expressing lymphoma utilizing the alpha-emitting radionuclide-conjugated monoclonal antibody 212Bi-anti-Tac. *Cancer Res* 54, 4362-4370 (1994)
109. Tan, C. & T. A. Waldmann: Proteasome inhibitor PS-341, a potential therapeutic agent for adult T-cell leukemia. *Cancer Res* 62, 1083-1086 (2002)
110. Zhang, M., Z. Zhang, C. K. Goldman, J. Janik & T. A. Waldmann: Combination therapy of adult T-cell leukemia xenografted mice with flavopiridol and an anti-CD25 monoclonal antibody. *Blood* (2004)
111. Williams, D. P., C. E. Snider, T. B. Strom & J. R. Murphy: Structure/function analysis of interleukin-2-toxin (DAB486-IL-2). Fragment B sequences required for the

delivery of fragment A to the cytosol of target cells. *J Biol Chem* 265, 11885-11889 (1990)
112. Kuhn, D. J. & Q. P. Dou: Direct Inhibition of Interleukin-2 Receptor alpha-Mediated Signaling Pathway Induces G1 Arrest and Apoptosis in Human Head-and-Neck Cancer Cells. *J. Cell Biochem* . Accepted (2005)
113. Kuroki, J., M. Hirokawa, A. Kitabayashi, M. Lee, T. Horiuchi, Y. Kawabata & A. B. Miura: Cell-permeable ceramide inhibits the growth of B lymphoma Raji cells lacking TNF-alpha-receptors by inducing G0/G1 arrest but not apoptosis: a new model for dissecting cell-cycle arrest and apoptosis. *Leukemia* 10, 1950-1958 (1996)
114. Ketley, N. J., P. D. Allen, S. M. Kelsey & A. C. Newland: Modulation of idarubicin-induced apoptosis in human acute myeloid leukemia blasts by all-trans retinoic acid, 1,25(OH)2 vitamin D3, and granulocyte-macrophage colony-stimulating factor. *Blood* 90, 4578-4587 (1997)
115. Ketley, N. J., P. D. Allen, S. M. Kelsey & A. C. Newland: Mechanisms of resistance to apoptosis in human AML blasts: the role of differentiation-induced perturbations of cell-cycle checkpoints. *Leukemia* 14, 620-628 (2000)
116. Isaacs, R. J., S. L. Davies, M. I. Sandri, C. Redwood, N. J. Wells & I. D. Hickson: Physiological regulation of eukaryotic topoisomerase II. *Biochim Biophys Acta* 1400, 121-137 (1998)
117. Gligorov, J. & J. P. Lotz: Preclinical pharmacology of the taxanes: implications of the differences. *Oncologist* 9 Suppl 2, 3-8 (2004)
118. Nichols, J., F. Foss, T. M. Kuzel, C. F. LeMaistre, L. Platanias, M. J. Ratain, A. Rook, M. Saleh & G. Schwartz: Interleukin-2 fusion protein: an investigational therapy for interleukin-2 receptor expressing malignancies. *Eur J Cancer* 33 Suppl 1, S34-36 (1997)
119. Saleh, M. N., C. F. LeMaistre, T. M. Kuzel, F. Foss, L. C. Platanias, G. Schwartz, M. Ratain, A. Rook, C. O. Freytes, F. Craig, J. Reuben, M. W. Sams & J. C. Nichols: Antitumor activity of DAB389IL-2 fusion toxin in mycosis fungoides. *J Am Acad Dermatol* 39, 63-73 (1998)
120. LeMaistre, C. F., M. N. Saleh, T. M. Kuzel, F. Foss, L. C. Platanias, G. Schwartz, M. Ratain, A. Rook, C. O. Freytes, F. Craig, J. Reuben & J. C. Nichols: Phase I trial of a ligand fusion-protein (DAB389IL-2) in lymphomas expressing the receptor for interleukin-2. *Blood* 91, 399-405 (1998)
121. Keppler-Hafkemeyer, A., R. J. Kreitman & I. Pastan: Apoptosis induced by immunotoxins used in the treatment of hematologic malignancies. *Int J Cancer* 87, 86-94 (2000)
122. Kreitman, R. J., W. H. Wilson, J. D. White, M. Stetler-Stevenson, E. S. Jaffe, S. Giardina, T. A. Waldmann & I. Pastan: Phase I trial of recombinant immunotoxin anti-Tac(Fv)-PE38 (LMB-2) in patients with hematologic malignancies. *J Clin Oncol* 18, 1622-1636 (2000)
123. Chaudhary, V. K., C. Queen, R. P. Junghans, T. A. Waldmann, D. J. FitzGerald & I. Pastan: A recombinant immunotoxin consisting of two antibody variable domains fused to Pseudomonas exotoxin. *Nature* 339, 394-397 (1989)
124. Chaudhary, V. K., M. G. Gallo, D. J. FitzGerald & I. Pastan: A recombinant single-chain immunotoxin composed of anti-Tac variable regions and a truncated diphtheria toxin. *Proc Natl Acad Sci U S A* 87, 9491-9494 (1990)
125. Batra, J. K., D. FitzGerald, M. Gately, V. K. Chaudhary & I. Pastan: Anti-Tac(Fv)-PE40, a single chain antibody Pseudomonas fusion protein directed at interleukin 2 receptor bearing cells. *J Biol Chem* 265, 15198-15202 (1990)
126. Robbins, D. H., I. Margulies, M. Stetler-Stevenson & R. J. Kreitman: Hairy cell leukemia, a B-cell neoplasm that is particularly sensitive to the cytotoxic effect of anti-Tac(Fv)-PE38 (LMB-2). *Clin Cancer Res* 6, 693-700 (2000)
127. Kozak, R. W., D. P. Fitzgerald, R. W. Atcher, C. K. Goldman, D. L. Nelson, O. A. Gansow, I. Pastan & T. A. Waldmann: Selective elimination in vitro of alloresponsive T cells to human transplantation antigens by toxin or radionuclide conjugated anti-IL-2 receptor (Tac) monoclonal antibody. *J Immunol* 144, 3417-3423 (1990)
128. Waldmann, T. A., J. D. White, J. A. Carrasquillo, J. C. Reynolds, C. H. Paik, O. A. Gansow, M. W. Brechbiel, E. S. Jaffe, T. A. Fleisher, C. K. Goldman & et al.: Radioimmunotherapy of interleukin-2R alpha-expressing adult T-cell leukemia with Yttrium-90-labeled anti-Tac. *Blood* 86, 4063-4075 (1995)
129. Adams, J., M. Behnke, S. Chen, A. A. Cruickshank, L. R. Dick, L. Grenier, J. M. Klunder, Y. T. Ma, L. Plamondon & R. L. Stein: Potent and selective inhibitors of the proteasome: dipeptidyl boronic acids. *Bioorg Med Chem Lett* 8, 333-338 (1998)
130. Orlowski, R. Z.: The role of the ubiquitin-proteasome pathway in apoptosis. *Cell Death Differ* 6, 303-313 (1999)
131. Li, B. & Q. P. Dou: Bax degradation by the ubiquitin/proteasome-dependent pathway: involvement in tumor survival and progression. *Proc Natl Acad Sci U S A* 97, 3850-3855 (2000)
132. An, B., R. H. Goldfarb, R. Siman & Q. P. Dou: Novel dipeptidyl proteasome inhibitors overcome Bcl-2 protective function and selectively accumulate the cyclin-dependent kinase inhibitor p27 and induce apoptosis in transformed, but not normal, human fibroblasts. *Cell Death Differ* 5, 1062-1075 (1998)
133. Won, K. A. & S. I. Reed: Activation of cyclin E/CDK2 is coupled to site-specific autophosphorylation and ubiquitin-dependent degradation of cyclin E. *Embo J* 15, 4182-4193 (1996)
134. Glotzer, M., A. W. Murray & M. W. Kirschner: Cyclin is degraded by the ubiquitin pathway. *Nature* 349, 132-138 (1991)
135. Aggarwal, B. B.: Nuclear factor-kappaB: the enemy within. *Cancer Cell* 6, 203-208 (2004)
136. Krappmann, D., F. G. Wulczyn & C. Scheidereit: Different mechanisms control signal-induced degradation and basal turnover of the NF-kappaB inhibitor IkappaB alpha in vivo. *Embo J* 15, 6716-6726 (1996)
137. Morgan, D. O.: Principles of CDK regulation. *Nature* 374, 131-134 (1995)
138. Sherr, C. J.: G1 phase progression: cycling on cue. *Cell* 79, 551-555 (1994)

Key Words: Interleukin-2, Interleukin-2 Receptor, Interleukin-2 Receptor Alpha, Monoclonal Antibodies, Chemotherapy, Radiotherapy, Cancer

Send correspondence to: Dr. Q. Ping Dou, Karmanos Cancer Institute at Wayne State University, 640.01 HWCRC, 4100 John R, Detroit, Michigan, 48201. Tel: 313-966-0641, Fax 313-993-0193; Alt. Fax 313-966-7368; E-mail: doup@karmanos.org

http://www.bioscience.org/current/vol10.htm