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# Successful immunotherapy to malignant cells with monoclonal antibody to suppressor T cells

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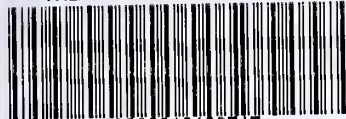
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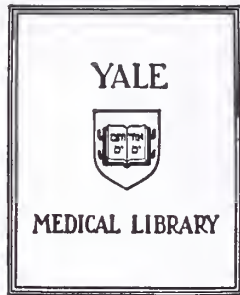
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SUCCESSFUL IMMUNOTHERAPY TO MALIGNANT CELLS WITH  
MONOCLONAL ANTIBODY TO SUPPRESSOR T CELLS

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Andres Felipe Rodriguez

1988







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Successful Immunotherapy to Malignant Cells with  
Monoclonal Antibody to Suppressor T Cells

A Thesis Submitted to the Yale University  
School of Medicine in Partial Fulfillment  
of the Requirements for the Degree of  
Doctor of Medicine

by

Andrés Felipe Rodríguez

1988




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## ACKNOWLEDGEMENTS

I would like to thank the following people without whom this thesis could not have been completed:

Dr. Patrick Flood, my thesis advisor, who allowed me to learn from my mistakes but was always present when I needed help;

Miss Pamela Reuter for her invaluable technical assistance and for guarding my mice and cell lines from other researchers;

and Miss Cristina Bengoa for her assistance in proofreading and finalizing this manuscript.

Finally, but most important, I would like to dedicate this thesis to my parents for the love and support they have provided me throughout the years.



## ABSTRACT

### Successful Immunotherapy to Malignant Cells with Monoclonal Antibody to Suppressor T Cells

Andrés Felipe Rodriguez

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Potent suppression of the growth of the progressive 31-52 methylcholanthrene-induced fibrosarcoma was observed in syngeneic C3H/HeN mice following treatment with a rat monoclonal antibody (mAb) raised against suppressor-effector T cells. Treatment of tumor-bearing animals with as little as 10ug of purified mAb 14-12 three times a week for four weeks lead to a significant decrease in tumor growth. The protective effects were not witnessed when mAb Y-3Ag, a rat antibody of identical isotype as the 14-12 hybridoma but of unknown antigen specificity, was used. Animals which resist a challenge of 31-52 tumor by treatment with mAb 14-12 show no macroscopic evidence of tumor relapse greater than 4 months after termination of treatment. Treatment with mAb 14-12 was shown to interfere with the activity of Ts cells and not other effector T cell populations. It is concluded that mAb 14-12 enhances tumor resistance in C3H/HeN mice by eliminating tumor-associated suppressor T cells, and this enhancement leads to increased tumor specific resistance.

Another phenomenon, that of augmentation of tumor growth, was also noted in mice treated with mAb 14-12 if low doses were used or when tumor growth was observed to progress poorly in control animals. Several possible explanations are discussed.



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## INTRODUCTION

The immune response to malignant cells is very complex. Despite the demonstration in tumor-bearing animals of immune reactivity to autochthonous tumor antigens (85) and the existence of a number of immune mechanisms which react to and eliminate tumor cells (3), some tumors continue to grow and eventually kill the host. The progressive growth of tumor cells in otherwise immunocompetent syngeneic animals has been attributed to a number of intricate tumor and host factors that lead to a breakdown of a normally competent immune response. One well documented method of escaping immunosurveillance is the tumor-induced generation of suppressor T (Ts) cells (4,5,8,11,12,13,22,29,30,31,53,54, 61,67,88,89,96,97,99,104,105). Tumor-associated Ts cell activity has been shown in a large number of tumor systems (reviewed in 67), and it has been postulated that reduction or elimination of Ts cells is critical for generating tumor rejection mechanisms in vivo (5).

While a number of agents which reduce or eliminate suppressor T cell activity in vivo have been used with varying success in the treatment of malignant disorders (10,16,29,36,44,89), these agents have other effects on the immune system as well. In the best study to date on the selective elimination of suppressor T cells, anti-I-J'



antiserum (36) or monoclonal antibody (10) was used to selectively eliminate suppressor T cell activity in tumor resistance studies with highly significant results. However, it has subsequently been shown that the I-J determinant is expressed on antigen-presenting cells (68), contrasuppressor T cells (Tcs; 115), T augmenting cells (76) and even subsets of helper T cells (Th; 57,101). Therefore, the selective role of suppressor T cells in enhancing tumor growth could not be determined in these studies.

The purpose of the current research was to study how the activity of Ts cells can be selectively manipulated to increase the immune response to malignantly transformed cells. It is hypothesized that treatment with a monoclonal antibody (mAb) specific for Ts cells and their soluble factors (TsF) will result in an enhanced immune response and rejection of the tumor. The antibody used in this study, mAb 14-12, was generated by immunization of rats with purified suppressor T cell factor from mice. This antibody has been shown to be specific for suppressor effector T cells and to block the biological activity of these cells in vitro (19) and in vivo (20,21,88).



## REVIEW OF LITERATURE

In 1908, the German bacteriologist Paul Ehrlich postulated that cancer cells arise frequently, they bear antigens on their surface membranes which could be detected as foreign by the host, and the immune system may be able to reject most of these cells (14). Based on Ehrlich's hypotheses, Thomas later formulated the theory of immune surveillance (106) which predicts that if cancer cells are immunogenic, they should induce an immune response in the host. Tumors that do form must then result from an immunoresistant cancer or an ineffective immune response. It is generally accepted that many tumors may escape immunosurveillance by suppression of the immune response. What follows is a brief overview of tumor immunology and a review of the role of suppressor T (Ts) cells.

### Antigenicity of Tumors and the Nature of Host Immunity

The fact that tumors possess surface antigens (tumor-associated antigen, TAA) against which an immunocompetent host can mount an immune response was first shown by Foley (27). The initial problem in tumor immunology was not in demonstrating an immune response to tumors, but rather in



attributing the immune response observed in animals with transplanted tumors to the tumor and not to histocompatibility differences between the tumor and the recipient. By 1957 with the availability of syngeneic animals and syngeneic tumor cell lines, the existence of specific cell-mediated immunity (CMI) to tumors was well established. In a landmark study, Prehn and Main (85) completely excised a carcinogen-induced tumor from an original host and transplanted varying numbers of tumor cells to syngeneic hosts. The smallest number of cells required to obtain tumors in all the recipient animals was determined. Retransplantation of this number of cells, called the LD<sub>100</sub>, into the original host usually resulted in no tumor growth, while control animals receiving the same number of cells developed tumors. They also found that the adoptive transfer of lymphocytes from the resistant animals to naive recipient animals resulted in specific transplantation resistance to the original tumor in recipient mice. This was later confirmed when it was shown that immunity to syngeneic tumors could be transferred by lymphocytes but not with serum from immune donors (21).

In an elegant study, Algire et al. (1) demonstrated that CMI is more significant than humoral immunity in tumor rejection. He used diffusion chambers with pores that allowed passage of molecules but not cells. Tumor cells were placed in these chambers and implanted into immune





hosts. Under these conditions the tumor cells survived and multiplied even though antibody could be detected bound to the tumor surface. When the experiment was repeated with diffusion chambers that allowed passage of cells, as well as molecules, the tumor cells were rejected and the tumor mass was found to be infiltrated by host inflammatory cells.

Upon further examination, it has been found that while tumors of the hematolymphoid system are generally susceptible to both humoral and cellular immunity (56,114), most tumors are not susceptible to attack by antibody alone or by antibody and complement, but rather are only sensitive to attack by the elements of CMI--cytotoxic T (Tc) cells, killer (K) cells, natural killer (NK) cells and macrophages (56,114). It is not known whether all systems function against all tumor cells or whether some tumors selectively induce one type of cell-mediated immune response over another.

### Tumor Escape Mechanisms

As stated by North (70), the central question of tumor immunology is: Why do immunogenic tumors grow progressively in immunocompetent hosts? The fact that tumor cells can grow progressively and sometimes metastasize, despite the existence of immune responses capable of rejecting them,



implies that there exist some mechanism or mechanisms that prevent the antitumor response from being effective in vivo, and that these mechanisms are more effective at the local rather than systemic levels. Several different mechanisms of tumor escape have been observed in experimental tumors:

Tolerance--Circulating tumor antigen or tumor cells themselves may induce tolerance (80). This means that the immune system is rendered specifically unresponsive to these tumor antigens and the tumor can then continue to grow unimpeded. Tolerance in many antigen systems has been shown to be Ts cell-mediated (111).

Immunoselection--It has been postulated that tumors are unicellular in origin and that progression of a tumor occurs by sequential selection of variant mutant subpopulations which are more adapted to an environment (75). This theory, known as the Clonal Selection Theory, is based upon the fact that an essential characteristic of all malignant cells is genetic instability. The same process that resulted in the development of a neoplastic cell (i.e. mutation), allows a tumor to adapt to new environments. Thus, an ongoing immune response or external antitumor therapy may destroy many tumor cells, but allow resistant cells to "sneak through" and clonally expand. Many different mechanisms can make a tumor cell resistant; for



example, weak immunogenicity, lack of TAA, different metabolic sensitivities, or the ability to induce Ts cell.

Antigen Modulation--In the presence of an ongoing immune response, tumor cells may lose or hide their original antigens by endocytosis and express a new set of antigens or no antigen (77).

Immunostimulation--Prehn and co-workers (83,84) have proposed that weak immunological responses to either small doses of tumor cells or weakly immunogenic tumor antigens may result in stimulation rather than inhibition of tumor growth. The mechanism is not known but possibilities include tumor stimulation by lymphokines (released from lymphocytes), or the preferential activation of Ts cells. Also, some aspects of the humoral immune response against tumor cells may stimulate tumor cell proliferation rather than destruction. The stimulation response may be similar to the stimulation of thyroid cells by antithyroid antibodies in Grave's disease (43).

Blocking Mechanisms--A high local concentration of TAA in the main tumor mass and free circulating TAA may block action of free antitumor antibody and T lymphocytes (56). Secondly, noncytotoxic antibodies may bind to TAA and block cytotoxic responses or the induction of cytotoxic responses (45).

Immunosuppression--Many tumor bearing patients and animals typically exhibit a general suppression of immunity that is



dependent on the tumor load (41). The classic example of immunosuppressed cancer patients is those with Hodgkin's Disease. These patients demonstrate a potent suppression of CMI as evidenced by poor DTH responses and increased incidence of intracellular parasitic infections such as tuberculosis (42).

The immune suppression evident in tumor-bearing hosts could be mediated by a number of mechanisms. Given the key regulatory role of suppressor T cells (6), it seems likely that these cells are at least partially responsible for the progressive growth of immunogenic tumors. Increased suppressor cell function has been demonstrated in numerous types of human cancer (3): osteogenic sarcoma, bladder cell carcinoma, Hodgkin's lymphoma, multiple myeloma, acute leukemia, colon carcinoma, head and neck cancer, lung cancer, and melanoma. The subject of Ts cells favoring tumor growth will be discussed in more detail in the next section.

### Ts Cells and Tumor Growth

Despite extensive evidence for the existence of CMI against tumors, the immune system sometimes appears to ignore or respond weakly to antigenic stimuli delivered by autochthonous tumor cells. Ortiz de Landazuri and Herberman





noted that no cell-mediated immune response could be detected in tumor bearing hosts after a tumor had progressed beyond a certain size (79). However, if host lymphocytes were incubated in tissue culture media for 24 hours and then replaced in tumor bearing animals (TBA), a specific cytotoxic immune response could be detected against the tumor. These results suggested the in vivo suppression of cell-mediated immune responses to some tumors.

The concept that Ts cells are responsible for certain states of unresponsiveness seen with conventional antigen or tumor systems arose from the initial observations of Gershon and his colleagues (33,34). The fact that mice bearing a variety of established tumors possess or can generate Ts cells is well documented (reviewed in 67). However, the mere possession or generation of Ts cells does not by itself demonstrate that these cells are responsible for the progressive growth of immunogenic tumors. Direct evidence linking Ts cells with progressive tumor growth can only be obtained when it is shown that the generation of tumor-induced Ts cells results in both the down regulation of an active antitumor immune response and progressive tumor growth.

Toward this end, Fujimoto et al. (29) have shown that the immune response to tumors implants in immunized animals could be suppressed by the transfer of thymus or spleen cells but not with serum from TBA or with thymus or spleen



cells from normal animals. In this study the methylcholanthrene-induced sarcoma 1509a was used. It was found that when normal A/Jax mice received  $10^6$  tumor cells all mice died within 40 days. However, syngeneic mice that had been rendered highly immune to the tumor by multiple implantations and resections, rejected the tumor within 14 days. The rejection of the tumor could be significantly inhibited by passive transfer of thymus or spleen cells from TBA, but the inhibitory effect was abolished by pretreatment of cells with anti-thymocyte serum. Fujimoto's group has also shown that the inhibition of tumor rejection was dependent on the number of thymus or spleen cells transferred. Based on these results, it was hypothesized that there exist immunosuppressor cells in TBA which are T cells and are capable of inhibiting the antitumor immune response.

These results were later confirmed by Berendt and North (4) and Dye and North (11) in two other tumor systems--the meth A fibrosarcoma and P815 mastocytoma, respectively. Immune mice were obtained by causing the regression of established tumors by injection of tumors with bacterial endotoxin or Corynebacterium parvum. It was found that the passive transfer of tumor-sensitized T cells from immune mice failed to cause regression of established tumors in immunocompetent mice, but caused the complete and permanent regression of established tumors if the recipient mice had



been made T cell deficient by thymectomy, lethal radiation , and bone marrow salvage (TXB). Tumor regression in TXB recipients could be blocked by the additional transfer of lymphocytes from nonimmune TBA, but not from normal animals. Successful transfer of tumor immunity has also been demonstrated in recipients which have been pretreated with cyclophosphamide (69) or sublethal radiation (46,71,72,103). Both procedures are known to decrease Ts cell population.

North and colleagues (73,74) have further characterized the nature of tumor-induced Ts cell generation by showing that TBA acquire T cells that can cause regression of small tumors in recipients on passive transfer during early growth of the tumor (between days 6 and 9). In later tumor growth, effector T cell population decreases and the appearance of T cells that can suppress adoptive immunity against established tumors begins. In contrast, Fujimoto's group has shown that Ts cells are generated within 24 hours of tumor implant (30).

Similar results were obtained by Treves and co-workers (108,109,110) and Umiel and Trainin (112). They found that spleen cells from C57/BL6 mice bearing the 3LL lung tumor could inhibit growth of the same tumor in recipient mice if the transferred cells were obtained after the injection of tumor cells but before the appearance of the tumor in the donor mice. If the spleen cells were obtained after the appearance of tumors in the donor animals, then transfer



resulted in enhanced tumor growth, as well as an increase in the number of lung metastases (112). After excision of the tumor in the donor animal, spleen cells quickly lost the ability to augment tumor growth (109). Treatment with anti-theta serum and complement, to deplete spleen cells of T cells, resulted in cells that could no longer augment tumor growth in recipient animals (110). It was further found that the supernatant of incubated immune spleen cells could, by itself, on transfer enhance tumor growth; however, if immune spleen cells were depleted of T cells and then incubated, the supernatant did not enhance tumor growth. Supernatant from immune spleen cells depleted of B cells and macrophages maintained the ability to enhance tumor growth (108).

The onset of tumor immunity has been associated with the presence of helper T cells (Th; 17,18) and cytolytic T cells (Tc; 60). Fernandez-Cruz and colleagues have shown that tumor immunity may be transferred against established murine sarcoma tumors by the selective transfer of Th cells to immunocompetent tumor-bearing recipients. North and associates have shown that the onset of immunity in the P815 mastocytoma/TXB recipient system was immediately preceded by the production of Tc cells which was significantly greater than the production observed in immunocompetent TBA or in TXB animals which also received T cells from nonimmune TBA, There was no Tc cell production observed in TXB tumor





bearing controls. Dye and North (11) also correlated the failure of tumor regression in TXB animals receiving transfer of nonimmune as well as immune T cells with the decreased production of Tc cells.

The presence of Ts cells capable of inhibiting the immune response against malignant cells has also been demonstrated in humans. In patients with osteogenic sarcoma, it has been shown that the peripheral blood lymphocytes of patients without detectable tumor specific cytotoxic activity in vitro contain a population of lymphocytes which mediate tumor specific cytotoxicity and a population of Ts cells that inhibit the in vitro activity of the cytotoxic cells.

Based on the results discussed above and that of numerous other groups (5,8,13,22,31,53,54,61,67,89,96,97,98,99,104,105), it has been concluded that, in many tumor systems, Ts cells develop which are capable of inhibiting effector T cell populations and allowing the progressive growth of immunogenic tumors.

### Mechanism of Ts Cell Induction

Exactly how tumors induce the generation of Ts cell is not known. A few possible mechanisms have been demonstrated in experimental models and are discussed below.



The ultraviolet (UV) light-induced spontaneous tumor system has been extensively studied and found to be associated with the generation of Ts cells. UV-induced tumors are highly antigenic and usually rejected in normal mice, however, UV-irradiated mice fail to reject these tumors (53). Kripke and Fisher (22,54) and Daynes and colleagues (8,99) demonstrated that the susceptibility of UV-treated mice to UV-induced tumors is an immunological phenomenon mediated by lymphocytes. Further study by Spellman and Daynes subsequently showed that UV-treated mice possess Ts cells, which appear prior to tumor implantation, can transfer susceptibility to UV-induced tumors (97), and are capable of specifically inhibiting antitumor immune responses (98).

An explanation for the appearance of Ts cells prior to tumor cells has been proposed by Greene et al. (40). They have demonstrated that UV radiation affects I-A<sup>+</sup> antigen presenting cells (APC) and prevents ordered antigen presentation. The defect in APC function is thought to lead to Ts cell generation. It is also possible that I-A<sup>-</sup> APC exist which are UV-resistant and preferentially present to Ts cells, or that UV radiation alters antigens, such that when presented by APC, Ts cells are preferentially induced. The importance of antigen presentation will be further discussed below.



Another experimental mechanism is the intravenous administration of soluble tumor antigens. Since the shedding of TAA into the peripheral circulation is known to occur (80), this may represent, at least in part, the mechanism by which progressive tumors activate Ts cells. The condition of "chronic antigen stimulation" may lead to the activation of Ts cells that will result in down regulation of an antitumor immune response and tolerance. Teleologically, it has been suggested that this mechanism may have evolved to prevent autoimmune disease; however, in tumor-bearing animals and patients this signal may result in a situation which favors tumor growth (3).

As mentioned previously inappropriate presentation of tumor antigens by APC has been suggested as a mechanism of Ts cell induction. It has been shown that macrophages treated with anti-I-A antibody (macrophages are I-A<sup>+</sup>) exclusively generate Ts (80). Based on this observation and those in the UV light tumor system, it was proposed that MHC class II-restricted antigen presentation is important in the induction of Th but not Ts cells, and further, it was proposed that Ts cells may have less strict requirements than Th cells about the context in which they recognize antigen (9,80). This means that tumor antigen presented to the immune system by class II<sup>-</sup> APC could result in the preferential activation of Ts cells.



## Mechanism of Ts Cell Action

In some systems Ts cells that inhibit the rejection of tumors in immunized mice have been shown to be specific (12) and produce tumor-specific soluble factors with immunosuppressive activity (T suppressor factor, TsF; 38). It has been demonstrated that suppression, by Ts cells and their factors, in conventional systems occurs through an interference with the development of Th cells specific for the antigen or directly by interaction with Th or B cells (6,35,100). Although the mechanisms are not clear, Ts cells also interfere with Tc cell (24), NK cell (47) and macrophage (86) activity, and probably that of inflammatory cells (i.e. CD4<sup>+</sup> T cells involved in inflammation; 59). It is likely that tumor-induced Ts cells down regulate the immune response to tumors in a similar fashion.

The important role of Ts cells in the regulation of the host immune response to tumor antigen suggests that these cells are reasonable targets to block tumor-mediated suppression. This thought has led to the study of tumor therapy with anti-Ts cell protocols, which will be discussed in the next section.

It should be noted that there also exist in TBA non-T suppressor cell which are capable of inhibiting immune responses (reviewed in 5,67). These cells, which include macrophages and B cells, are capable of nonspecific





suppression of the immune system and may be important in some tumor systems.

### Immunotherapy in the Treatment of Cancer

The current treatment of cancer primarily consists of surgery, radiation therapy, and/or chemotherapy. Any side effects of treatment on lymphoid cells and the immune response have been accepted as necessary. Immunotherapy or augmentation of the host's own immune response is now considered the emerging fourth modality of cancer treatment (71,78).

#### Nonspecific Immunostimulation

Various methods of nonspecifically stimulating an animal's or patient's immune response have been tried. For example, inoculation of tumor with the bacterial antigens, BCG (an attenuated form of mycobacterium) and C. parvum leads to an immune response which results in the destruction of intracellular antigen and also an increase in the number and activity of macrophage and NK cells (71). Increased numbers of these cells often leads to enhanced tumor destruction as well. Unfortunately, these methods are



limited to accessible tumors and have met with minimal clinical success (90).

### Antitumor Monoclonal Antibodies

Since the development of the hybridoma technique by Kohler and Milstein in 1975 (51), a more promising anticancer approach has been the use of tumor-specific mAbs (reviewed in 58). Monoclonal antibodies directed against TAA can be used through their direct antitumor effect or through the targeting of chemotherapeutic or other cytotoxic agents with mAb-antitumor agent complexes. Clinical successes with mAb therapy have been achieved but so far have also been limited and difficult to reproduce.

### Adoptive Immunotherapy

Numerous attempts to specifically increase the intensity of antitumor responses have been made with adoptive immunotherapy (reviewed in 90,92). In this approach tumor-sensitized lymphocytes are transferred to a tumor-bearing host and mediate either directly or indirectly an immune response against the tumor. Although transfer of immune cells usually inhibits the growth of tumor implants, tumor growth has sometimes been enhanced (104). In some cases the transfer of cells results in autoimmune disease (7).



Until recently, most successes with adoptive therapy have either been with tumor implants or small tumor burdens, or depended on the elimination of host T<sub>s</sub> cells prior to transfer (91). For example, North and his associates have shown that combination therapy with cyclophosphamide and immune spleen cells caused the complete regression of established tumors in all mice, while spleen cells alone had no effect (69). It was also noted that cyclophosphamide alone caused a temporary halt in tumor growth.

#### Adoptive Immunotherapy with Interleukin-2 (IL-2)

With the discovery of T cell Growth Factor or IL-2 (94), adoptive immunotherapy of established tumors has been more successful. IL-2 which is known to amplify and activate lymphocytes (lymphokine-activated killer cells, LAK) is a most promising anticancer agent. In rodent experiments, combination of LAK cells and purified IL-2, has been shown to cause regression of established pulmonary and hepatic metastases for a variety of tumors (55,63,64). Based on these models, Rosenberg and colleagues (91) have used autologous LAK cells with IL-2 (in culture and in vivo) to produce partial to complete remission in approximately forty-five percent of patients with advanced cancer. It should be noted that these patients had failed standard therapy. Best results were obtained with the following



cancers: metastatic melanoma, colorectal carcinoma, renal cell cancer, and adenocarcinoma of the lung--all of which are fairly resistant to therapy with conventional agents.

### Anti-Ts cell Therapy

Ts cells inhibit the efficacy of host effector responses against certain tumors. Thus decreasing or eliminating Ts cells and their factors is a rational approach to the treatment of malignancies suspected of inducing immune suppression.

It is likely, in fact, that the success of certain conventional treatment regimens partly depends on elimination of Ts cell activity. Cyclophosphamide (Cytosan) is one of the most effective chemotherapeutic agents available and has been shown to decrease Ts cell activity (44,69). Irradiation, another clinically useful modality, is also known to potentiate certain in vivo immune responses by diminishing Ts cell activity (46,71,110). Ts cells may have a short lifespan and/or be dependent on tumor load. If this is the case, then tumor excision (surgery) may decrease Ts cells and result in more effective antitumor responses (3).

The importance of the T cell system as a whole to host defenses against neoplasia, as well as microorganisms, should be emphasized. Regimens that nonselectively impair





overall T cell function may result in life threatening situations due to immune compromise, such as overwhelming infection, and not necessarily increase survival of a tumor-bearing host.

One study which has shown the importance of selectively inhibiting Ts cell activity was conducted by Greene et al. (39). Anti-thymocyte serum (ATS) when administered to syngeneic mice between day 0 and day 1 after tumor implantation enhanced tumor growth, while ATS given between day 3 and day 10 reduced tumor growth. This discrepancy was thought to be due to nonspecific T cell destruction and a reflection of which T cell population predominated at each point of tumor growth, Th, Tc, or Ts. Other studies have tested the following treatments which may preferentially eliminate Ts cells but still affect other immune cell populations: splenectomy (16), splenic irradiation (16), low dose cyclophosphamide (44,69), low dose irradiation (72), adult thymectomy (89), and anti-I-J sera and antibody (10,36).

Using the most selective of these regimens, monoclonal anti-I-J antibody, Greene's group has shown that daily treatment with nanogram doses i.v. results in the significant suppression of growth of the MCA-induced S1590a and SaI syngeneic tumors in A/Jax mice (10). Histological exam of treated TBA revealed substantial infiltration and necrosis of tumors compared to control TBA. It was further



shown that spleens from TBA could no longer enhance tumor growth on transfer to immune tumor-bearing recipients if the cells were first treated with anti-I-J antibody.

When the I-J subregion was initially characterized and Greene's study was published it was believed that the subregion was uniquely associated with Ts cells and factors (66,81,82,102). However, it has subsequently been shown that the subregion serologically defines other T cell regulatory activities as well. It is now known that I-J determinants are expressed on Th cells (57,101), augmenting T cells (76), contrasuppressor T cells (Tcs,116), and macrophage (68), as well as Ts cells.

Further studies with anti-I-J reagents have shown that leukemic cell growth is enhanced and mortality increases in syngeneic mice (57). It is thought that the enhancement of tumor growth is due to interference of a humoral response which is under the control of an I-J<sup>+</sup> Th cell. Recall that humoral immunity is important in host defenses against leukemia. Other groups have also shown that I-J<sup>+</sup> cells are necessary for the adoptive transfer of syngeneic tumor immunity (28).

The initial excitement over therapy with anti-I-J antibody has now passed, but a monoclonal antibody raised specifically against T cell suppressor factor has been described recently by Ferguson et al. (19). This mAb called 14-12 is able to block the activity of Ts cells and



TsF of various genetic and antigenic specificities in vitro (19), and is known not to bind to Th, Tc, Tcs, APC, or B cells by negative or positive selection techniques (Horvat, B., Flood, P.M. unpublished results). The current study was designed to evaluate the effect of mAb 14-12 on tumor growth in vivo.



## MATERIALS AND METHODS

Mice: 6-10 week old C3H/HeN (mammary tumor virus negative, MTV-) mice from colonies of germ-free derived specific pathogen free (SPF) animals were obtained from the Frederick Cancer Research Center, NCI, Frederick, Maryland. In all therapeutic experiments each group consisted of at least 5 mice.

Fibrosarcoma Line: The fibrosarcoma 3152-PRO is a nonmetastatic tumor which kills by infiltration of vital organs and tumor load. This tumor was previously induced in UV-irradiated C3H/HeN (MTV-) mice by subcutaneous injection of 3-methylcholanthrene under the ventral non-UV exposed skin (65). Tumor cells were maintained in vitro in Dulbecco's Modified Eagles Medium (DMEM; GIBCO, Grand Island, NY) supplemented with 10% heat inactivated fetal calf serum (FCS), 2 mM l-glutamine, 50U/ml penicillin, and 50 ug/ml streptomycin (GIBCO).

Preparation and Transplantation of 3152-PRO: For tumor challenges, cells were grown in vitro and taken during late log phase growth. Tumor cells were washed three times in Hank's Balanced Salt Solution (HBSS; Flow Laboratories, McLean, Va). Cells ( $1 \times 10^6$ ) were injected subcutaneously in 0.2 ml of Phosphate-buffered saline (PBS; pH 7.2) on the





back of challenged animals. All mice were injected at the same time and then randomly divided into experimental groups.

Measurement of Tumor Size: Tumor growth was measured macroscopically using a vernier caliper by measuring the longest diameter of two perpendicular measurements of the tumor mass to calculate tumor area. This method has been shown to correlate with tumor weight (36). The tumors were examined once a week independently by two investigators during the course of the experiments. Animals with tumors greater than  $1\text{mm}^3$  (the size at which a mass can be easily detected by palpation of skin at the site of tumor injection) designated tumor bearing animals (TBA).

Monoclonal Antibodies: The monoclonal anti-suppressor T cell antibody was previously generated as described by Ferguson et al. (19). Briefly, rats were immunized with 100ug of purified T suppressor factor (TsF) isolated from the serum of mice hyperimmunized to sheep red blood cells (SRBC). Spleen cells from rats immunized with TsF were fused with Y-3Ag 1.2.3, a hypoxanthine-aminopterin-thymidine (HAT)-sensitive (i.e. hypoxanthine guaninephosphoribosyl-transferase deficient) rat myeloma, producing an IgM monoclonal antibody of unknown specificity. Positive hybridomas were chosen based on their ability to bind



purified immunogen in an enzyme linked immunosorbent assay (ELISA), and cloned by limiting dilution.

The 14-12 monoclonal antibody (mAb) has been shown to be sensitive to and specific for suppressor effector T cells and factor of a number of different antigenic and genetic specificities (19). This antibody can be used to eliminate suppressor effector T cell activity in vivo (20,21,88). Antibody Y3Ag, produced by the myeloma parent used in the fusion to generate 14-12, has no demonstrable effect on suppressor T cells or factors (19). Antibody Y3Ag was used for control rat IgM in some experiments and was prepared identically to serum-free mAb 14-12.

Preparation of mAb 14-12 for injection: Purified serum-free antibody was obtained by growing 14-12 cells in vitro in Iscove's serum free medium (GIBCO, Grand Island, New York) containing only gentamicin, insulin, progesterone, transferrin, and trace metals as described by Mosier (62). MAb 14-12 was seeded at  $10^7$  cells/ml and grown to confluency in medium. Cell supernatant was cleared by centrifugation and passage through a sterile 0.2  $\mu$ m filter (Millipore/Continental Water Systems, Bedford, MA). The antibody was precipitated by a 45% cut of saturated ammonium sulfate (SAS). The precipitate was redissolved in PBS and dialyzed against PBS. Purified antibody showed only an IgM heavy chain and a K light chain in SDS PAGE under reducing conditions (gel not shown). Antibody concentration was



determined by ELISA and optical density spectrophotometry at wavelength 280nm.

Antibody from ascites fluid was prepared by injecting 10<sup>7</sup> hybridoma cells intraperitoneally into mice which had been pretreated with pristane 3 weeks prior. Twenty-four hours prior to injection with hybridoma cells the animals were injected with 0.1 ml of rabbit anti-thymocyte serum subcutaneously. On the day of injection mice received 500 rads of irradiation and were then injected with hybridoma cells. Ascites fluid was harvested after 5-7 days and was purified in an identical fashion to the serum-free mAb.

Treatment with mAb 14-12: In therapy experiments, injections of 0.05 ml containing a given dose of antibody were injected either intravenously, intraperitoneally, or intradermally at the site of tumor injection three times a week, one time a week, or once as noted.

Antigens: 2,4,6-Trinitrobenzene sulfonate (TNBS) was obtained from Sigma Chemical Company (St. Louis, MO). 2,4,6-Trinitro-1-chlorobenzene or Picryl Chloride (PCl) was obtained from the Eastman Chemical Company (Smithtown, NY).

Preparation of Spleen Cells: Spleens were aseptically removed from mice killed by cervical dislocation. Spleen cells were gently teased apart from fibrous tissue in HBSS and centrifuged at 500 x G for 5 minutes. Spleen cells were



washed twice with HBSS and resuspended in HBSS. Further preparation was as described below.

Preparation of Contrasuppressor T cell-deficient Spleen

Cells: The I-J encoded determinant of contrasuppressor T cells is recognized by the monoclonal anti-I-J reagent 4B-11 which distinguishes from the I-J determinant found on Ts cells (116). Anti-I-J 4B-11 was kindly provided by Dr. Charles Janeway, Yale University School of Medicine, New Haven, CT. Spleen cells ( $1 \times 10^7$  cells/ml) prepared as described above were incubated with anti-I-J (diluted 1:1000 in HBSS) at room temperature for 30 minutes. After centrifugation at  $500 \times G$  for 5 minutes, spleen cells were resuspended in HBSS. Rabbit serum (Pel-Freez, Rogers, AK; diluted 1:10 in HBSS), used as a source of complement, was then added in an equal volume to the spleen cells. The preparation was incubated at  $37^\circ C$  for 30 minutes. Cells were then washed three times with HBSS and resuspended in HBSS.

Preparation of Hapten-modified Spleen Cells: Spleen cells prepared as described above were trinitrophenylated (TNP-) at room temperature for 10 minutes in PBS (pH 7.2) containing 3mM TNBS. The TNP-spleen cells were washed three times with HBSS and resuspended in HBSS.

Adoptive Transfer of Contact Sensitivity and Treatment with

mAb 14-12: Donor mice were immunized with 5% PCl in





ethanol:acetone (3:1) by painting shaved abdomens (0.20 ml) and placing a drop on each paw (2). Four days later these mice were sacrificed and spleens were removed and treated with a monoclonal anti-I-J reagent (described above) to eliminate contrasuppressor T cells which would affect Ts cell activity (88). Anti-I-J-treated spleen cells ( $3 \times 10^7$ ) were transferred by intravenous injection to a second set of mice which had been primed on the previous day with 14-12 (10ug) or PBS (control). Recipients were immunized immediately with 1% PCl in olive oil on the right ear. Contact sensitivity response was measured 24 hours later as described below.

Tolerance Induction and Treatment with mAb 14-12: On day 0 mice were pretreated with 1) nothing, 2) PBS, or 3) 14-12 (10 ug). On day 1 some groups of mice were immunized with  $2 \times 10^7$  TNP-spleen cells intravenously. Four days later all mice were immunized with 1% PCl in olive oil on the right ear. Contact sensitivity response was measured 24 hours later as described below.

Measurement of Contact Sensitivity: The contact sensitivity response was determined by measuring ear swelling with an engineer's micrometer. Each ear was measured in triplicate in a "blind" protocol. All contact sensitivity values are expressed as units of swelling as determined by the differ-



ence of the left ear (unchallenged) vs the right ear (challenged). 1 unit =  $10^{-9}$  cm.

Statistical Analysis: Data are reported as Mean values  $\pm$  S.E.M. Analysis of data was performed using the Student's t test with 95% confidence intervals.



## RESULTS

### Treatment with MAb 14-12 Affects the Progressive growth of the Murine Fibrosarcoma 3152-PRO.

#### MAb 14-12 Inhibits Tumor Growth and Decreases Tumor Incidence.

Normal age-matched C3H/HeN mice were transplanted with 3152-PRO tumor cells as described above. Immediately after transplantation, animals were put into a therapeutic regimen of three times a week, one time a week, or single injections of 100 ug of mAb 14-12 (prepared from serum-free (SF) media). The results in Table I show the ability of mAb 14-12 to inhibit tumor growth. Control animals injected with 3152-PRO tumor cells all developed sizeable tumors within 3 weeks. Animals transplanted with 3152-PRO tumor cells and injected with single doses or weekly injections of mAb 14-12 showed little or no reduction of tumor incidence. However, animals transplanted with tumor cells and given three injections a week of mAb 14-12 showed a greatly reduced incidence of tumor. This effect was not observed in animals given identical therapeutic regimens of mAb Y3Ag, a nonspecific rat isotype matched control antibody.



In similar experiments the rate of tumor growth was quantified weekly by physical measurement of tumors. Figure 1 shows that injections 3 times a week of 10 ug of mAb 14-12 (prepared from ascites) significantly inhibited (week 1  $p < 0.001$ , weeks 2 and 4  $p < 0.01$ , week 3  $p < 0.02$ ) tumor growth as early as day 7 after tumor inoculation. This effect persisted for at least 28 days which was the duration of therapy. In this experiment mAb therapy resulted in greatly reduced incidence of tumor as well. All control animals (n=5) developed palpable tumors by week 1; whereas only one treatment animal (n=5) had developed a tumor by week 4. Animals that resisted a challenge of 3152-PRO tumor by treatment showed no evidence of tumor even four months after the termination of treatment.

The Regimen and Route of Administration of MAb 14-12 is Critical.

Experiments were also done to test various methods of administering the mAb 14-12. As previously mentioned, most significant inhibition of tumor growth was obtained with therapeutic regimens of repeated injections 3 times a week as opposed to once a week or single injections (Table I; Figure 2). As shown in figure 2 the route of immunization was also found to be critical. Best results were obtained with intraperitoneal (i.p.) or intratumor (i.t.) injections.





Treatment with mAb 14-12 by i.p. or i.t. routes three times a week resulted in the complete inhibition of tumor growth. Again all control animals (n=5) developed palpable tumors by week 1, but no treatment animal showed evidence of tumor for greater than 4 months after termination of treatment. Intravenous (i.v.) injection also significantly decreased tumor growth by week 4 (wk 3  $p < 0.05$ , week 4  $p < 0.01$ ), but was not as effective as i.p. or i.t. routes. Four of five animals in the i.v. group developed tumors by week 2.

#### The Effect of MAb 14-12 is Dose-related.

The results in Figure 3 reveal that animals treated with mAb 14-12 (prepared from SF media) 3 times a week demonstrate a dose response. The maximum dose tested was 10 ug/injection. This dose was chosen because it had been effective in previous studies of mAb 14-12 (20). Treatment with 10 ug/injection was most effective and was statistically significant ( $p < 0.01$ ) by week 3. Treatment groups receiving smaller doses of mAb 14-12 had subsequently larger mean tumor sizes. At lowest doses of mAb the treatment groups developed larger tumors than those that developed in control animals (discussed in next section). It should be pointed out that although tumors grew slower in treatment groups (except group receiving 0.01 ug/injection), by week 3 all mice had developed tumors.



### Augmentation of Tumor Growth.

As mentioned above, another interesting phenomenon may be seen in the dose response curve (Figure 3). Groups of animals treated with low doses of mAb 14-12 (i.e. 0.1 ug/injection or less) actually had slightly larger mean tumor sizes and higher tumor growth rates than did the control group given identical regimens of saline injections. In fact, by day 28 all animals (n=5) in the group receiving 0.01 ug/injection had succumbed to tumors that on the average were significantly larger than control tumors ( $p < 0.01$ ).

The phenomenon of tumor augmentation was also seen when the 3152-PRO tumor grew poorly in the control animals. For example, Table II shows that no animals in either control or treatment groups had developed tumors by week 1. By week 2 tumors began to develop in treatment animals (2/5), but no control animal developed a tumor until week 5. Mean tumor size was significantly different at the  $p < 0.02$  level by week 5. It is interesting to note that in this experiment, treatment with mAb 14-12 not only increased the mean tumor size of the group of animals, but also the number of tumor-bearing animals per group by week 5 (Control 1/5; Treatment 5/5).



Monoclonal Antibody 14-12 Selectively Blocks Ts Cell Action.The Suppression of the Adoptive Transfer of Contact Sensitivity to PCL is Blocked in Animals Treated with MAb 14-12.

It has previously been shown that animals painted with picryl chloride (PCL) show a strong contact sensitivity (CS) reaction on reapplication of the same or a cross-reacting agent such as trinitrophenol (TNP; 87). This antigen-specific CS reaction can be adoptively transferred by immune spleen cells into naive recipients. It is also known that the transfer of CS can be suppressed by an antigen-specific Ts factor, TNP-TsF (93). The transfer of CS requires at least 2 distinct T cell populations, an Ly1+ I-J Vicia villosa nonadherent (VV-) DTH effector T ( $T_{DTH}$ ) cell, and an Ly1+ I-J+ VV adherent (VV+) contrasuppressor T (Tcs) cell (48,88). Depletion of either cell type blocks the adoptive transfer. The  $T_{DTH}$  effector cell can only transfer antigen-specific CS to naive animals in the presence of the Tcs cell, which "protects" the effector cell from host suppressor mechanisms (i.e. TNP-TsF), or in the absence of Tcs cells if host suppressor mechanisms are compromised. For example, treatment of animals with cyclophosphamide, which has been shown to overcome Ts cell-mediated



mechanisms (48), allows the  $T_{DTH}$  cell alone to transfer CS (88).

Figure 4 shows the results of an adoptive transfer study in which normal age-matched C3H/HeN mice were pretreated with PBS or 10 ug mAb 14-12 on day 0. On day 1 groups C and D received syngeneic immune spleen cells from mice which had been sensitized 4 days earlier by skin painting. In order to selectively eliminate Tcs cells the immune spleen cells were treated with anti-I-J prior to transfer. CS response was determined as described in Materials and Methods. It can be seen that the mice which were pretreated with mAb 14-12 and received anti-I-J treated immune cells (group D) developed a CS response which was significantly different ( $p < 0.001$ ) from mice which received no immune cells (Groups A and B) or no mAb (Group C). Nonspecific effects of mAb 14-12 were controlled for in group B which received antibody but no immune spleen cells. Treatment with antibody alone had no effect on CS response when compared to the control animals (Group A). The fact that elimination of Tcs cells and treatment with mAb 14-12 resulted in the transfer of CS suggests that mAb 14-12 interferes with the action or generation of Ts cells and does not affect other immune cell populations such as the  $T_{DTH}$  effector cell.





Tolerance Induction to TNP is Blocked and Converted to an Immunogenic Signal in Animals Treated with MAb 14-12.

Tolerance to an antigen such as TNP can be induced by injecting syngeneic hapten-conjugated spleen cells (TNP-SC) intravenously (111). The tolerizing signal is very potent and can be given even after subcutaneous injection (which immunizes for a CS response rather than tolerance). It has been well established that this tolerizing signal is mediated by antigen specific Ts (111).

Figure 5 reveals that C3H/HeN mice, which were injected with mAb 14-12 prior to i.v. TNP-SC transfer, developed a CS response rather than tolerance. The response observed was significantly different ( $p \ll 0.001$ ) from that observed in controls. Animals receiving a tolerance-inducing protocol but no mAb 14-12 (Groups D and E) did develop tolerance as evidenced by CS responses which were similar to control animals (Groups A and B). Group C which consisted of animals primed with mAb 14-12 but not immunized with TNP-SC again reveals that mAb 14-12 had no nonspecific effects on the CS response. These results show that treatment with mAb 14-12 converts a normally tolerogenic signal (i.v. TNP-SC) to an immunogenic one. The hypothesis that mAb 14-12 selectively interferes with Ts cell action is again supported by this data.



## DISCUSSION

The present study has shown that in vivo treatment with mAb 14-12 specifically interferes with Ts cell activity and not with the activity of effector T cell populations. MAb treatment allowed the transfer of a contact sensitivity response to PC1 with Tcs cell-deficient immunized spleen cells. Under these conditions the transfer of CS, which is mediated by a DTH effector T cell, is normally blocked by Ts cells in untreated recipients. Treatment with mAb 14-12 also converted a Ts cell-mediated tolerogenic signal (i.v. TNP-SC) to an immunogenic one which again requires an effector T cell. In both cases, mAb 14-12 alone had no nonspecific augmenting effect on the immune response. Other studies have shown that mAb 14-12 does not interact with Th, Tc, APC, or B cells in vitro (Horvat, B., Flood, P.M. unpublished results). These results suggest that mAb 14-12 can alter Ts cell activity in vivo with profound consequences on immunity to many antigenic systems including tumors.

The effect of mAb 14-12 on tumor growth was studied and it was shown that treatment with this antibody can significantly inhibit the growth of a highly progressive murine fibrosarcoma in syngeneic animals. As expected, more frequent administration of antibody has a more potent effect



on tumor growth than does less frequent administration. The effect of treatment with mAb 14-12 was also found to be dose-related with 10 ug three times a week being most effective. The effectiveness of this dose correlates well with subsequent in vivo studies of mAb 14-12 (20,21). Although 10 ug was the highest dose tested in this study, other studies have not found higher doses to have a greater effect (20). The fact that the inhibitory effect of purified mAb 14-12 was found to be dose-related and dependent on the frequency of administration strongly suggests that the effect of treatment was antibody-mediated and not related to other factors such as stress of injection, immunological or disease state of the animals, or the diluent.

The route of administration was also found to effect outcome of treatment. Intratumor and i.p. routes were most effective, while i.v. therapy was less effective. This result may be artifactual as i.v. injection into the retroorbital plexus is technically a more difficult form of administration, and thus, a full dose of antibody may not have been delivered at every injection. Another possible explanation is that i.v. administration results in a more rapid clearance of antibody and therefore has a diminished effect.

The mechanism by which mAb 14-12 interferes with the suppressor cell circuit is uncertain but several mechanisms



are possible. The fact that mAb 14-12 reacts with Ts cells and TsF of different antigenic and genetic specificities (19), combined with the knowledge that Ts cells have been shown to act through an anti-idiotypic mechanism in some systems (24), suggests that the antibody may interact with cells through an idiotype mechanism. The antibody may bind to Ts cells, TsF, or a subfactor and prevent the interaction of these cells or molecules with Th cells or other effector T cells. Alternatively, mAb 14-12 may bind to immature suppressor cells and prevent the differentiation to functional Ts cells. As mAb 14-12 is an IgM molecule, if bound to Ts cells, it could also initiate complement mediated lysis.

The results presented here, together with those of earlier experiments have demonstrated the importance of Ts cells and factor in the regulation of the immune response to tumors and the usefulness of an antibody specific for Ts cells in the study of regulatory T cell circuits. The success of tumor therapy with mAb 14-12 supports the hypothesis that selective interference with Ts cells allows a host to mount a more effective immune response resulting in increased tumor resistance.

Additional studies with mAb 14-12 have recently shown that, used in combination with IL-2, it could cause 60 to 90 percent reduction of established pulmonary metastases of the weakly immunogenic MCA-induced fibrosarcoma 106 (49).





Combination therapy was more effective than either agent alone. The finding that mAb 14-12 had no detectable antitumor activity when used by itself was thought to reflect an inadequate immune response to the tumor used even without suppression by Ts cells.

Application of therapy to other tumors with mAbs similar to 14-12 needs to be carefully studied. In the present study an IgM mAb to Ts cells was used; however for several reasons an IgG antibody would probably be more effective. IgG antibodies have higher affinity for idiotype<sup>+</sup> material than do IgM antibodies. IgG molecules are more able to cross endothelial barriers (due to smaller size), and thus would have access to more body compartments. While both molecules are able to fix complement, only IgG can activate antibody-dependent cell-mediated cytotoxicity responses (113). IgG anti-suppressor mAbs have recently been synthesized and are currently undergoing investigation with conventional antigen and tumor systems.

The findings of this study are potentially very exciting. As more is learned about immunoregulation and tumor immunity it becomes more possible to treat cancer. Immunotherapy may be more effective if Ts cells as well as tumor cells are targeted by the treatment. Eventually tumor therapy may include a mAb directed at Ts cells in humans, or the combination of such an antibody with other antitumor agents or with the transfer of immune cells and IL-2.



Several problems that may interfere with the use of mAb 14-12 or another similar antibody against Ts cells should be mentioned. These include the development of autoimmunity due to lack of Ts cells to down-regulate the immune response. MAb 14-12 blocks Ts cells of various specificities. But if an antibody could be produced that reacted to antigen- or tumor-specific Ts cells only, then autoimmunity would not be a problem.

Another potential problem arises with the use of murine mAbs in humans. The development of allergic responses characterized by fever, chills, dyspnea, and anaphylactoid reactions has been reported in some patients who have been treated with murine antibodies (56). In these patients circulating antibodies to mouse Ig (mIg) could be detected and were sometimes associated with diminished clinical response (56,95). Thus, in addition to allergic responses, it appears that antibodies develop to mouse Ig which inhibit therapeutic efficacy. Several methods could potentially be implemented to avoid these problems--development of human mAb, induction of tolerance to mouse Ig, or the development of mAb coupled to cytotoxic agents (cells interacting with mIg would then be destroyed and no immune response to mIg would develop). Another solution may be the use of hybrid antibodies. Recombinant technology has been used to synthesize mAbs that combine the specificity of mouse



V-regions with human C-regions (50). In theory, these hybrid antibodies should be less immunogenic.

In the present study another phenomenon, that of augmentation of tumor growth, was also observed in mice treated with mAb 14-12. This effect was observed with low doses of mAb (i.e. 0.1 ug or less) and when tumors grew poorly in controls. This result may have been spurious, but the facts that the effect was dose-related in some cases and all animals within a treatment group behaved the same suggests that some common factor was affecting each member of the group. Experiments were designed to control for the stress of injection, stock of mAb, and the age of animals; however, some important factors were not controlled--immune or disease state of animals and phenotypic alterations in tumor cells.

Disease state of animals can effect immune function including tumor rejection. Animals were specific pathogen free when obtained from the supplier, but at any time during experiments some animals may have been infected by a virus or other pathogen. Diseased animals may be unable to mount a significant immune response even in the absence of Ts cells. Perhaps macrophages or other immune cell populations have decreased function in diseased animals. Non-infected control animals may then mount relatively more effective immune responses even in the presence of Ts cells. The



concept of an insufficiently activated immune response will be further discussed below.

With long term passage of tumors phenotypic alterations in tumor cells occur. In this study, tumor cells were always harvested during late log phase growth, but the number of serial passages from cryopreserved samples was not controlled. It is possible that the tumor became less immunogenic during serial passage. Decreased immunogenicity would make the tumor cells less susceptible to immune rejection. In experimental systems, increasing the immunogenicity of tumors has been shown to result in increased Tcs cell populations and enhanced tumor rejection (23,26). Although speculative, the Tcs cells are thought to be important in tumor rejection because they protect effector T cells from suppression by Ts cells (26,32,37). If decreasing immunogenicity results in decreased Tcs cell populations, the immune response to a less immunogenic tumor may be more susceptible to suppression by Ts cells. Phenotypic alterations may also result in tumors that can cause more potent induction of Ts cells. MAb 14-12 may not be as effective at blocking Ts cell activity under these conditions.

Other explanations are possible for the tumor augmentation observed with mAb 14-12. Low doses of mAb may stimulate Ts cells while higher doses inhibit their activity. Under certain conditions anti-idiotypic reagents





are able to stimulate lymphocyte clones expressing the idiotype. This has been shown in some B cell systems (15,107). Perhaps low doses of mAb or the induction of Ts cells by tumors with impaired viability or in suboptimal doses may result in conditions such that an anti-idiotypic reaction between mAb 14-12 and Ts cells augments Ts cell activity.

Also, Ts cells may have both positive and negative effects on tumor growth. In addition to mediating suppression, Ts cells may secrete lymphocyte inhibitory factors (such as lymphotoxin) or tumor growth factors. It has been shown that lymphotoxin, which is usually cytotoxic, can stimulate protein synthesis in target cells at low doses (52). Under certain conditions (e.g. suboptimal tumor challenge or low dose of antibody), mAb 14-12 may block cell-mediated suppression but not secretion of such factors. These conditions could result in increased tumor growth.

An alternative explanation for the observed tumor augmentation effect is that while mAb 14-12 blocks suppression, it does not directly activate the immune system. MAb 14-12 treatment may be more effective when combined with immune stimulation regimens. In the future it will be interesting to study whether the effect of tumor augmentation can be abolished if IL-2 (with or without LAK



cell transfer) is used in combination with an anti-suppressor cell antibody.

Regardless of the mechanism by which tumor growth was augmented in some experiments, this phenomenon needs to be investigated in more detail. Additional study may help to further characterize regulatory T cell circuits and the immune response to tumors.



## REFERENCES

1. Algire, G.H., Weaver, J.M., Prehn, R.T. Growth of cells in vivo in diffusion chambers. I. Survival of homografts in immunized mice. J. Natl. Cancer Inst. 15:493-507, 1954.
2. Asherson, G.L., Ptak, W. Contact and delayed hypersensitivity in the mouse. I. Active sensitization and passive transfer. Immunol. 15:405-416, 1968.
3. Balch, C.M. Immune responses to tumor antigens and their clinical applications. Surgery 100:562-571, 1986.
4. Berendt, M.J., North, R.J. T-cell-mediated suppression of anti-tumor immunity: An explanation for progressive growth of an immunogenic tumor. J. Exp. Med. 151:69-80, 1980.
5. Broder, S., Waldman, T.A. The suppressor cell network in cancer. New Eng. J. Med. 299:1335-1341.
6. Cantor, H., Gershon, R. K. Immunological circuits: Cellular composition. Fed. Proc. 38:2058-2064, 1979.
7. Cohen, J.R., Globerson, A., Feldman, M. Lymphoid cells sensitized in vitro against allogenic or syngeneic fibroblasts produce effects in vitro and in vivo. Transpl. Proc. 3:892-894, 1971.
8. Daynes, R.A., Spellman, C.W. Evidence for the generation of suppressor cells by ultraviolet radiation. Cell. Immunol. 31:182-187, 1977.
9. Drebin, J.A., Perry, L.L., Carter, R., Greene, M.I. Regulation of the immune response to antigens on the malignant cell surface. Springer Sem. Immunopath. 5:175-192, 1982.
10. Drebin, J.A., Wallenbaugh, C., Schatten, S., Benacerraf, B. Inhibition of tumor growth by monoclonal anti-I-J antibodies. J. Immunol. 130:506-509, 1983.
11. Dye, E.S., North, R.J. T cell mediated immunosuppression as an obstacle to adoptive immunotherapy of the P815 mastocytoma and its metastases. J. Exp. Med. 154:1033-1042, 1981.
12. Dye, E.S., North, R.J., Mills, C.D. Mechanisms of anti-tumor action of Corynebacterium parvum I. Potentiated



- tumor-specific immunity and its therapeutic limitations. J. Exp. Med. 154:609-620, 1981.
13. Eggers, A.E., Wunderlich, J.R. Suppressor cells in tumor-bearing mice capable of nonspecific blocking of in vitro immunization against transplantation antigens. J. Immunol. 114:1554-1556, 1975.
  14. Ehrlich, P. The collected papers of Paul Ehrlich, vol. II. p. 550. Pergamon Press, London, 1957.
  15. Eichmann, K. Idiotypic Suppression I. Influence of the dose and of the effector functions of anti-idiotypic antibody on the production of an idiotypic. Eur. J. Immunol. 4:296-302, 1974.
  16. Enker, W.E., Jacobitz, J.L. In vivo splenic irradiation eradicates suppressor cells causing the regression and inhibition of established tumor. Int. J. Cancer 25:819-825, 1980.
  17. Fernandez-Cruz, E, Feldman, J.D. Immunotherapy of progressive tumors with T cell subsets. In The Potential Role of T Cells in Cancer Therapy, eds. Fefer, A., Goldstein, A. pp. 53-63. Raven Press, New York, 1982.
  18. Fernandez-Cruz, E., Woda, B.A., Feldman, J.D. Elimination of syngeneic sarcomas in rats by a subset of T lymphocytes. J. Exp. Med. 152:823-841, 1980.
  19. Ferguson, T.A., Beaman, K.D., Iverson, G.M. Isolation and characterization of a T-suppressor factor by using a monoclonal antibody. J. Immunol. 134:3163-3171, 1982.
  20. Ferguson, T.A., Ptak, W., Iverson, G.M., Flood, P.M. The role of suppression in immunoregulation: In vivo analysis using a monoclonal antibody to T-suppressor factors. Submitted for publication.
  21. Ferguson, T.A., Rodriguez, A., Iverson, G.M., Flood, P.M. Infectious and non-infectious tolerance are blocked by a monoclonal antibody to T-suppressor factors. Submitted for publication.
  22. Fisher, M.S., Kripke, M.L. Systemic alteration induced in mice by ultraviolet light irradiation and its relationship to ultraviolet carcinogenesis. Proc. Natl. Acad. Sci. USA 74:1688-1692, 1977.
  23. Flood, P.M., Friedman, A., Horvat, B., Reuter, P., Rodriguez, A., Ptak, W. Contrasuppression and Tumor Rejection. Immunol. Letters. In press.





24. Flood, P.M., Kripke, M.L., Rowley, D.A., Schreiber, H. Suppression of tumor rejection by autologous anti-idiotypic immunity. Proc. Natl. Acad. Sci. USA 77:2209-2213, 1980.
25. Flood, P.M., Ptak, W., Green, D.R. Mechanism of action of a T-suppressor factor (TsF) in contact sensitivity: The T cell target for TsF activity in adoptive transfer of immunity is not the effector cell. J. Immunol. 137:1829-1835, 1986.
26. Flood, P.M., Schreiber, H., Ron, Y. Protective immunity to progressive tumors can be induced by antigen presented on regressor tumors. J. Immunol. 138:3573-3579, 1987.
27. Foley, E.J. Antigenic properties of methylcholanthren-induced tumors in mice of the strain of origin. Cancer Res. 13:835-837, 1953.
28. Frelinger, J.A., Lukasewycz, O., Hill, S.W., Hibbler, F. In Ir Genes and Ia Antigens, ed. McDevitt, HO. pp. 417-421. Academic Press, New York, 1978. Cited by Meruelo, D., et al. Proc. Natl. Acad. Sci. USA 77:2178-2182, 1980.
29. Fujimoto, S., Greene, M.I., Sehon, A.H. Regulation of the immune response to tumor antigens. I. Immunosuppressor cells in tumor-bearing hosts. J. Immunol. 116:791-799, 1976.
30. Fujimoto, S., Greene, M.I., Sehon, A.H. Regulation of the immune response to tumor antigens. II. The nature of immunosuppressor cells in tumor-bearing hosts. J. Immunol. 116:800-806, 1976.
31. Fujimoto, S., Mutsuzawa, T., Nakagawa, K., Tada, T. Cellular interaction between cytotoxic and suppressor T cells against syngeneic tumors in the mouse. Cell. Immunol. 38:378-387, 1978.
32. Gershon, R.K., Eardley, D.D., Durum, S., Green, D.R., Shen, F.W., Yanauchi, K., Cantor, H., Murphy, D.B. Contrasuppression: A novel immunoregulatory activity. J. Exp. Med. 153:1533-1546, 1981.
33. Gershon, R.K., Kondo, K. Infectious immunological tolerance. Immunol. 21:903-914, 1971.
34. Gershon, R.K., Mokyry, M.B., Mitchell, M.S. Activation of suppressor T cells by tumor cells and specific antibody. Nature 250:594-596, 1974.



35. Greene, M.I., Benacerraf, B. Studies on hapten-specific T cell immunity and suppression. Immunol. Rev. 50:1-24, 1980.
36. Greene, M.I., Dorf, M.E., Pierres, M., Benacerraf, B. Reduction of syngeneic tumor growth by anti-I-J alloantiserum. Proc. Natl. Acad. Sci. USA 74:5118-5121, 1977.
37. Green, D.R., Eardley, D.D., Kimura, A., Murphy, D.B., Yamauchi, K., Gershon, R.K. Immunoregulatory circuits which modulate responsiveness to suppressor cell signals: Characterization of an effector cell in the contra-suppressor circuit. Eur. J. Immunol. 11:973-980, 1981.
38. Greene, M.I., Fujimoto, S., Sehon, A.H. Regulation of the immune response to tumor antigens. III. Characterization of thymic suppressor factor(s) produced by tumor bearing hosts. J. Immunol. 119:757-764, 1977.
39. Greene, M.I., Perry, L.L., Benacerraf, B. Regulation of immune response to tumor antigens. V. Modulation of suppressor-T-cell activity in vivo. Am. J. Path. 95:159-169, 1979.
40. Greene, M.I., Sy, M.S., Kripke, M., Benacerraf, B. Impairment of antigen presenting cell function by ultraviolet radiation. Proc. Natl. Acad. Sci. USA 76:6591-6595, 1979.
41. Harrison's Principles of Internal Medicine, 11th ed. Braunwald, E., Isselbacher, K.J., Petersdorf, R.G., Wilson, J.D., Martin, J.B., Fauci, A.S., eds. Principles of Neoplasia, by Mendelsohn, J. p. 428. McGraw Hill Book Company, New York, 1987.
42. Harrison's Principles of Internal Medicine, 11th ed. Braunwald, E., Isselbacher, K.J., Petersdorf, R.G., Wilson, J.D., Martin, J.B., Fauci, A.S., eds. Hodgkin's Disease and the Lymphocytic Lymphomas, by DeVita, V.T., Ultmann, J.E. p. 1553. McGraw Hill Book Company, New York, 1987.
43. Harrison's Principles of Internal Medicine, 11th ed. Braunwald, E., Isselbacher, K.J., Petersdorf, R.G., Wilson, J.D., Martin, J.B., Fauci, A.S., eds. Diseases of the Thyroid, by Ingbar, S.H. p. 1743. McGraw Hill Book Company, New York, 1987.
44. Hellstrom, I., Hellstrom, K.E. Cyclophosphamide delays 3-methylcholanthrene sarcoma induction in mice. Nature 275:129-130, 1978.



45. Hellstrom, K.E., Hellstrom, I. Lymphocyte-mediated cytotoxicity and blocking serum activity to tumor antigens. Adv. Immunol. 18:209-277, 1974.
46. Hellstrom, K.E., Hellstrom, I., Kant, J.A., Tamerius, J.D. Regression and inhibition of sarcoma growth by interference with a radiosensitive T cell population. J. Exp. Med. 148:799-804, 1978.
47. Itoh, K., Tilden, A.B., Balch, C.M. Role of interleukin 2 and a serum suppressive factor on the induction of activated killer cells cytotoxic for autologous human melanoma cells. Cancer Res. 45:3173-3178, 1985.
48. Iverson, G.M., Ptak, W., Green, D.R., Gershon, R.K. The role of contrasuppression in the adoptive transfer of immunity. J. Exp. Med. 158:982-987, 1983.
49. Kim, B., Warnaka, P., Iverson, M., Imbembo, A.L. Immunotherapy of pulmonary metastases using monoclonal antibody to T-cell suppressor factor and interleukin 2. Arch. Surg. 122:1455-1459, 1987.
50. Kohler, G. Derivation and diversification of monoclonal antibodies. Science 233:1281-1286, 1986.
51. Kohler, G., Milstein, C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256:494-97, 1975.
52. Kolb, W.P., Granger, G.A. Lymphocyte in vitro cytotoxicity: Characterization of mouse lymphotoxin. Cell. Immunol. 1:122-132, 1970.
53. Kripke, M.L. Antigenicity of murine skin tumors induced by ultraviolet light. J. Natl. Cancer Inst. 53:1333-1336, 1974.
54. Kripke, M.L., Fisher, M.S. Immunological parameters of ultraviolet carcinogenesis. J. Natl. Cancer Inst. 57:211-215, 1976.
55. Lafreniere, R., Rosenberg, S.A. Successful immunotherapy of murine experimental hepatic metastases with lymphokine-activated killer cells and recombinant interleukin-2. Cancer Res. 45:3735-3741, 1985.
56. Levy, R. Biologicals for cancer treatment: Monoclonal antibodies. Hosp. Prac. 20:67-92, 1985.



57. Meruelo, D., Flieger, N., Smith, D., McDevitt, H.O. In vivo or in vitro treatments with anti-I-J alloantisera abolish immunity to AKR leukemia. Proc. Natl. Acad. Sci. USA 77:2178-2182, 1980.
58. Merz, B. Fine-tuned and loaded, monoclonals treat cancer. JAMA 256:1406-1412, 1986.
59. Miller, S.D., Calman, H.N. The induction of hapten-specific T cell tolerance by using hapten-modified lymphoid cells. I. Characteristics of tolerance induction. J. Immunol. 117:1519-1526, 1976.
60. Mills, C.D., North, R.J. Expression of passively transferred immunity against an established tumor depends on generation of cytolytic T cells in recipient: Inhibition of suppressor T cells. J. Exp. Med. 157:1448-1460, 1983.
61. Minami, A., Kasai, M., Mizushima, Y., Takeichi, N., Hosokawa, M., Kobayashi, H. Characterization of immunosuppressor cells in rats immunized with solubilized tumor-associated antigen prepared from a methylcholanthrene-induced fibrosarcoma. Cancer Res. 40:2129-2134, 1980.
62. Mosier, EE. Primary in vitro antibody responses by purified murine B lymphocytes in serum-free defined medium. J. Immunol. 127:1490-1493, 1981.
63. Mule, J.J., Shu, S., Rosenberg, S.A. The anti-tumor efficacy of lymphokine-activated killer cells and recombinant interleukin-2 in vivo. J. Immunol. 135:646-52, 1985.
64. Mule, J.J., Shu, S., Schwarz, S.L., Rosenberg, S.A. Adoptive immunotherapy of established pulmonary metastases with LAK cells and recombinant interleukin-2. Science 225:1487-1489, 1984.
65. Mullen, C.A., Urban, J.L., Van Waes, C., Rowley, D.A., Schreiber, H. Multiple cancers: tumor burden permits the outgrowth of other cancers. J. Exp. Med. 162:1665-1682, 1985.
66. Murphy, D.B., Herzenberg, L.A., Okumara, K., Herzenberg, L.A., McDevitt, H.O. A new I subregion (I-J) marked by a locus (Ia-4) controlling surface determinants on suppressor T lymphocytes. J. Exp. Med. 144:699-712, 1976.
67. Naor, D. Suppressor cells: Permitters and promoters of malignancy? Adv. Cancer Res. 29:45-125, 1979.





68. Niederhuber, J.E., Mayo, L, Schreffler, D.C. In Ir Genes and Ia antigens, ed McDevitt HO. pp. 393-404. Academic Press, New York, 1978. Cited by Meruelo, D., et al. Proc. Natl. Acad. Sci. USA 77:2178-2182, 1980.
69. North, R.J. Cyclophosphamide-facilitated adoptive immunotherapy of an established tumor depends on elimination of tumor-induced suppressor T cells. J. Exp. Med. 155:1063-1074, 1982.
70. North, R.J. The generation and down regulation of the antitumor immune response. Ly1+ suppressor T cells abridge the generation of Ly2+ effector T cells. In Progress in Immunology V. eds. Yamamura, Y., Tada, T. pp. 1239-1251. Academic Press Japan, Tokyo. 1983.
71. North, R.J. The murine immune response and its therapeutic manipulation. Adv. Immunol. 35:89-155, 1984.
72. North, R.J. Radiation-induced, immunologically mediated regression of an established tumor as an example of successful therapeutic immunomanipulation. Preferential elimination of suppressor T cells allows sustained production of effector T cells. J. Exp. Med. 164:1652-1666, 1986.
73. North, R.J., Bursucker, I. Generation and decay of the immune response to a progressive fibrosarcoma. I. Ly1+2- suppressor T cells down-regulate the generation of Ly1-2+ effector T cells. J. Exp. Med. 159:1295-1311, 1984.
74. North, R.J., Dye, E.S. Ly1+2- suppressor T cells down-regulate the generation of Ly1-2+ effector T cells during progressive growth of the P815 mastocytoma. Immunol. 54:47-56, 1985.
75. Nowell, P.C. The clonal evolution of tumor cell populations: Acquired genetic lability permits stepwise selection of variant sublines and underlies tumor progression. Science 194:23-28, 1976.
76. Ochi, A., Nonaka, M., Hayakawa, K., Okumura, K.O., Tada, T. Two loci in I-J subregion of the H-2 complex controlling molecules selectively expressed on suppressor and helper T cells. J. Immunol. 129:227-231, 1982.
77. Old, L.J., Stockert, E., Boyse, E.A., Kim, J.H. Antigenic modulation: Loss of TL antigen from cells exposed to TL antibody. Study of the phenomenon in vitro. J. Exp. Med. 127:523-539, 1968.



78. Oldham, R.K., Thurman, G.B., Talmadge, J.E., Stevenson, H.C., Foon, K.A. Lymphokines, monoclonal antibodies, and other biological response modifiers in the treatment of cancer. Cancer 54:2795-2806, 1984.
79. Ortiz de Landazuri, M., Herberman, R.B. In vivo activation of cellular immune response to Gross virus-induced lymphoma. J. Exp. Med. 136:969-983, 1972.
80. Perry, L.L., Greene, M.I. T cell subset interaction in the regulation of syngeneic tumor immunity. Fed. Proc. 40:39-44, 1981.
81. Pierres, M., Germain, R.N., Dorf, M.E., Benacerraf, B. Potentiation of a primary in vivo antibody response by alloantisera against gene products of the I region of the H-2 complex. Proc. Natl. Acad. Sci. USA 74:3975-3979, 1977.
82. Pierres, M., Germain, R.N., Dorf, M.E., Benacerraf, B. In vivo effects of anti-Ia alloantisera in elimination of specific suppression by in vivo administration of antisera specific for I-J controlled determinants. J. Exp. Med. 147:656-666, 1978.
83. Prehn, R.T. Do tumors grow because of the immune response of the host? Transpl. Rev. 28:34-42, 1976.
84. Prehn, R.T., Lappe, M.A. An immunostimulation theory of development. Transpl. Rev. 7:26-54, 1971.
85. Prehn, R.T., Main, J.M. Immunity to methylcholanthrene-induced sarcomas. J. Natl. Cancer Inst. 18:769-778, 1957.
86. Ptak, W., Gershon, R.K. Immunological agnosis: A state that derives from T-suppressor cell inhibition of antigen-presenting cells. Proc. Natl. Acad. Sci. USA 79:2645-2648, 1982.
87. Ptak, W., Gershon, R.K., Flood, P.M. Mechanisms of suppression in the transfer of contact sensitivity. Analysis of an I-J+ molecule required for Ly-2 suppressor cell activity. J. Exp. Med. 158:1822-1835, 1983.
88. Ptak, W., Green, D.R., Flood P. Cellular interactions in the adoptive transfer of contact sensitivity. Characterization of an antigen-nonspecific Vicia villosa adherent T cell needed for adoptive transfer into naive recipients. J. Immunol. 137:1822-1828, 1986.



89. Reinisch, C.J., Andrew, S.L., Schlossman, S.F. Suppressor cell regulation of tumor immunity: Abrogation by adult thymectomy. Proc. Natl. Acad. Sci. USA 74:2989-2992, 1977.
90. Rosenberg, S.A. Adoptive immunotherapy of cancer: Accomplishments and prospects. Cancer Treat. Rep. 68:233-255, 1984.
91. Rosenberg, S.A., Lotze, M.T., Muul, L.M., Leitman, S., Chang, A.E., Etinghausen, S.E., Matlory, Y.L., Skibber, J.M., Shiloni, E., Vetto, J.T., Seipp, C.A., Simpson, C., Reichart, C.M. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. New Eng. J. Med. 313:1485-1492, 1985.
92. Rosenberg, S.A., Terry, W.D. Passive immunotherapy of cancer in animals and man. Adv. Cancer Res. 25:323-394, 1973.
93. Rosenstien, R.W., Murray, J.H., Cone, R.E., Ptak, W., Iverson, G.M., Gershon, R.K. Isolation and partial characterization of antigen-specific T cell factor associated with the suppression of delayed-type hypersensitivity. Proc. Natl. Acad. Sci. USA 78:5821-5825, 1981.
94. Ruscetti, F.W., Morgan, D.A., Gallo, R.C. Functional and morphologic characterization of human T cells continuously grown in vitro. Immunol. 119:131-138, 1977.
95. Shawler, D.C., Bartholemew, R.M., Smith, L.M., Dillman, R.O. Human immune response to multiple injections of murine monoclonal IgG. J. Immunol. 135:1530-1535, 1985.
96. Small, M., Trainin, N. Separation of populations of sensitized lymphoid cells into fractions inhibiting and fractions enhancing syngeneic tumor growth in vivo. J. Immunol. 117:292-297, 1976.
97. Spellman, C.W., Daynes, R.A. Modification of immunological potential by ultraviolet radiation. II. Generation of suppressor cells in short term ultraviolet irradiated mice. Transpl. 24:120-126, 1977.
98. Spellman, C.W., Daynes, R.A. Ultraviolet light induced murine suppressor lymphocytes dictate specificity of anti-ultraviolet light tumor immune responses. Cell. Immunol. 38:25-34, 1978.



99. Spellman, C.W., Woodward, J.G., Daynes, R.A. Modification of immunological potential by ultraviolet radiation. I. Immune status of short term ultraviolet irradiated mice. Transpl. 24:112-119, 1977.
100. Tada, T., Okumara, K. The role of antigen-specific T cell factors in the immune response. Adv. Immunol. 28:1-87, 1979
101. Tada, T., Takemori, T., Okumara, K., Nanaka, M. Tokuhisa, T. Two distinct types of helper T cells involved in the secondary antibody response: Independent and synergistic effect of Ia- and Ia+ helper T cells. J. Exp. Med. 147:446-458, 1978.
102. Tada, T., Taniguchi, M., David, C.S. Properties of the antigen-specific suppressor T cell factor in the regulation of the antibody response of the mouse. IV. Special subregion assignment of the gene(s) that codes for the suppressive T cell factor in the H-2 histocompatibility complex. J. Exp. Med. 144:713-725, 1976.
103. Tada, T., Taniguchi, M., Okumara, K. Regulation of homocytotropic antibody formation in the rat. II. Effect of x-irradiation. J. Immunol. 106:1012-1018, 1971.
104. Takei, F., Levy, J.G., Kilburn, D.G. In vitro induction of cytotoxicity against syngeneic mastocytoma and its suppression by spleen and thymus cells from tumor-bearing mice. J. Immunol. 116:288-293, 1976.
105. Takei, F., Levy, J.G., Kilburn, D.G. Characterization of suppressor cells in mice-bearing syngeneic mastocytoma. J. Immunol. 118:412-417, 1977.
106. Thomas, L. Reactions to homologous tissue antigens in relationship to hypersensitivity. In Cellular and Humoral Aspects of the Hypersensitive States, ed. Lawrence, H.S. pp. 529-532. Hoerber-Harper, New York, 1959. Cited by Prehn, R.T. Transpl. Rev. 28:34-42, 1976.
107. Trenkner, E., Riblet, R. Induction of antiphosphorylcholine antibody formation by anti-idiotypic antibody. J. Exp. Med. 142:1121-1132., 1975.
108. Treves, A.J., Cohen, I.R., Feldman, M. Suppressor factor secreted by T-lymphocytes from tumor-bearing mice. J. Natl. Cancer. Inst. 57:409-414, 1976.





109. Treves, A.J., Cohen, I.R., Feldman, M. A syngeneic metastatic tumor model in mice: The natural immune response of the host and its manipulation. Isr. J. Med. Sci. 12:369-383, 1976.
110. Treves, A.J., Carnaud, C., Trainin, N., Feldman, M., Cohen, I.R. Enhancing T lymphocytes from tumor-bearing mice suppress host resistance to a syngeneic tumor. Eur. J. Immunol. 4:722-727, 1974.
111. Tsurufuji, M., Sy, M., Benacerraf, B. An antigen-specific signal is required for the activation of second-order suppressor T cells in the regulation of delayed-type hypersensitivity to 2,4,6-trinitrobenzene sulfonic acid. J. Exp. Med. 158:932-945, 1983.
112. Umiel, T., Trainin, N. Immunological enhancement of tumor growth by syngeneic thymus-derived lymphocytes. Transpl. 18:244-250, 1974.
113. Volk, W.A. Essentials of Medical Microbiology, 2nd ed. p. 315. J.B. Lippincott Co., Philadelphia, 1982.
114. Volk, W.A. Essentials of Medical Microbiology, 2nd ed. p. 320. J.B. Lippincott Co., Philadelphia, 1982.
115. Yamauchi, K., Green, D.R., Eardley, D.D., Murphy, D.B., Gershon, R.K. Immunoregulatory circuits that mediate responsiveness to suppressor cell signals: Failure of B10 mice to respond to suppressor factors can be overcome by quenching the contrasuppressor circuit. J. Exp. Med. 153:1547-1561, 1981.
116. Yamauchi, K., Taniguchi, M., Green, D.R., Gershon, R.K. The use of a monoclonal I-J specific antibody to distinguish cells in the feedback suppression circuit from those in the contrasuppression circuit. Immunogen. 16:551-588, 1982.
117. Yu, A., Watts, H., Jaffe, N., Parkman, R. Concomitant presence of tumor-specific cytotoxic and inhibitory lymphocytes in patients with osteogenic sarcoma. New Eng. J. Med. 297:121-127, 1977.



Table I. The effect of monoclonal antibody 14-12 and the control antibody Y3Ag on the in vivo growth of the murine fibrosarcoma 31-52P.

<u>Frequency of antibody treatment</u>	<u>Tumor Growth on Day 21 following tumor challenge (TBA/Challenged Animals)</u>	
	<u>14-12</u>	<u>Y3Ag</u>
Control (0)	10/10	10/10
1 total	10/10	10/10
1 inj/wk	9/10	10/10
3 inj/wk	2/10	10/10



Table II. The augmentation of tumor growth is seen in animals treated with mAb 14-12 when tumors grow poorly in controls.

<u>Time</u> (wks)	<u>Tumor Growth (TBA/Challenged Animals)</u> (Mean Tumor Area $\pm$ S.E.M.)	
	<u>Control</u>	<u>Treatment</u>
1	0 (0/5)	0 (0/5)
2	0 (0/5)	0.4 (2/5)
3	0 (0/5)	18 + 18 (2/5)
5	15 + 15 (1/5)	109 + 28 (5/5)







Figure 1.--The Inhibitory Effect of MAb 14-12 on the Growth of a Murine Fibrosarcoma in Syngeneic Mice. Growth curves of the MCA-induced 31-52PRO tumor in control (saline) and in treatment (10ug mAb 14-12 (ascites) i.p. 3X/wk) animals reveal the ability of mAb 14-12 to significantly reduce tumor growth as early as day 7 after tumor inoculation ( $p < 0.001$ ). This effect persisted for the duration of the experiment (wk 2 and 4  $p < 0.01$ ; wk 3  $p < 0.02$ ). All treated animals which resisted a challenge of tumor cells showed no evidence of tumor four months after termination of treatment. TBA/Challenged animals by week 4: Control 5/5, Treatment 1/5.

Mice were challenged with  $10^7$  cells 31-52PRO tumor cells s.q. on day 0 and tumor size was measured weekly by calculating tumor area. Tumor size for each group ( $n=5$ ) is expressed as mean tumor area  $\pm$  S.E.M. Statistical differences between experimental and control groups was established by student's t test.

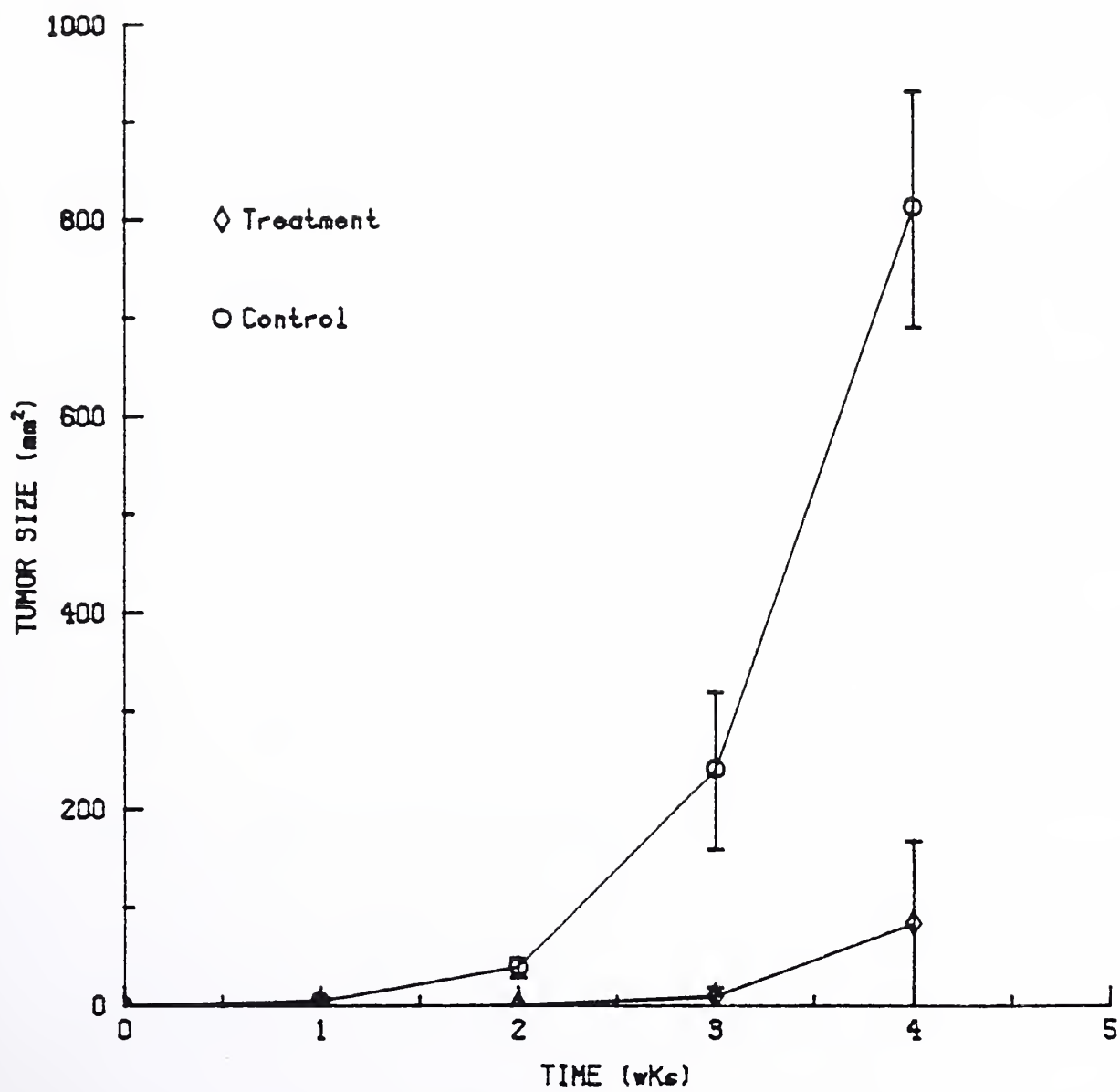






Figure 2.--The Route and Frequency of Administration of MAb 14-12 are Critical. The inhibitory effect of SF mAb 14-12 (10ug 3X/wk) on the growth of the MCA-induced 31-52PRO tumor was greatest when the antibody was administered i.p. or i.t. for 4 weeks (wk 2 p < 0.01; wk 3 p < 0.001; wk 4 p << 0.001). Treatment i.v. for 4 weeks (wk 4 p < 0.01) or i.p. for only one week (N.S.) also inhibited tumor growth but was not as effective. Animals which completely resisted a challenge of tumor cells by treatment showed no macroscopic evidence of tumor even 4 months after termination of treatment. TBA/Challenged animals by week 4: control 5/5, i.v. and i.p. (1 wk) 4/5, i.t. and i.p. (4 wks) 0/5. Note--Growth curves for the i.t.- and i.p.- (4 wks) treated animals are superimposed on the x-axis.

Mice were challenged with  $10^7$  cells 31-52PRO tumor cells s.q. on day 0 and tumor size was measured weekly by calculating tumor area. Tumor size for each group (n=5) is expressed as mean tumor area  $\pm$  S.E.M. Statistical differences between experimental and control groups was established by student's t test.

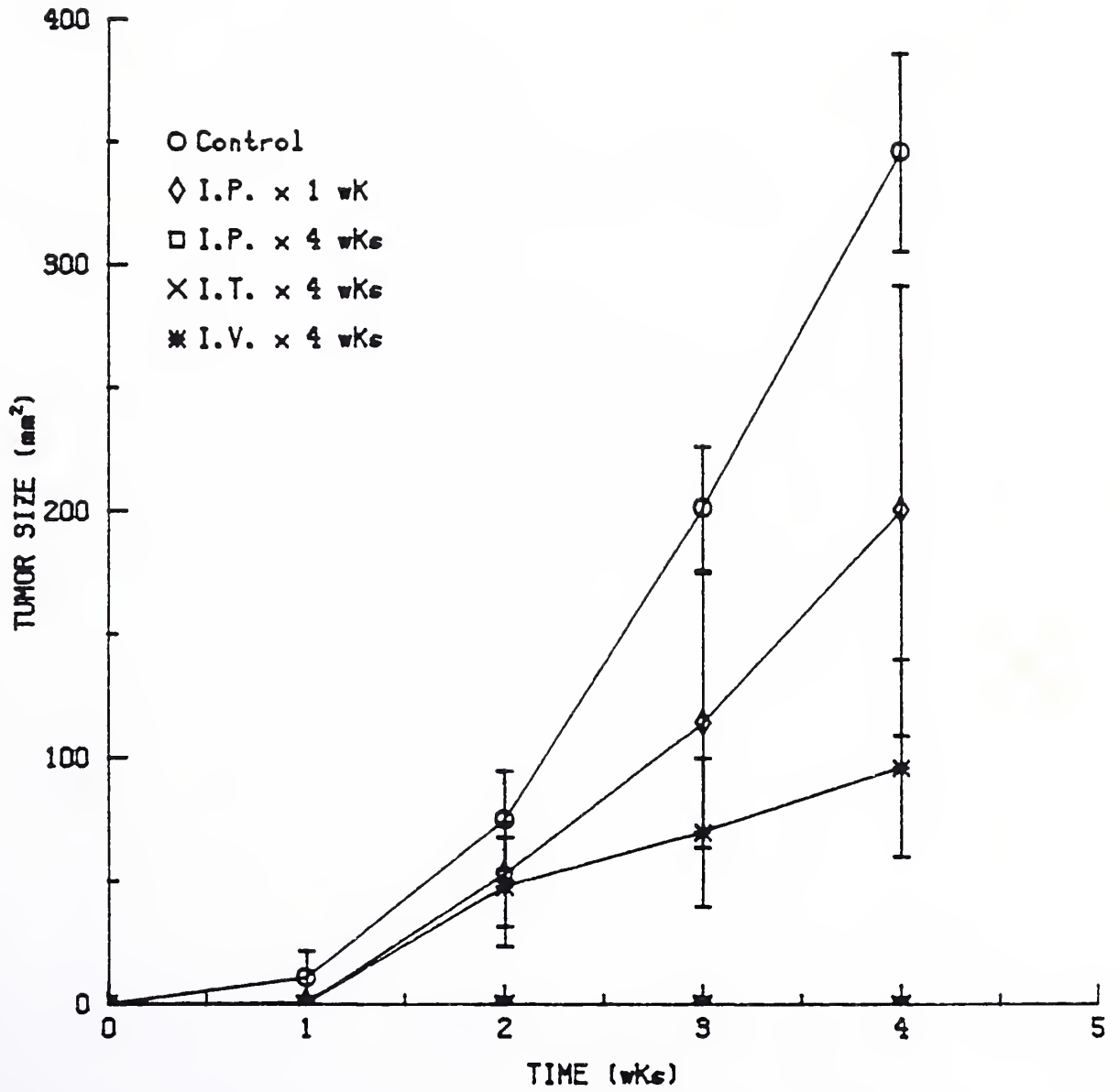


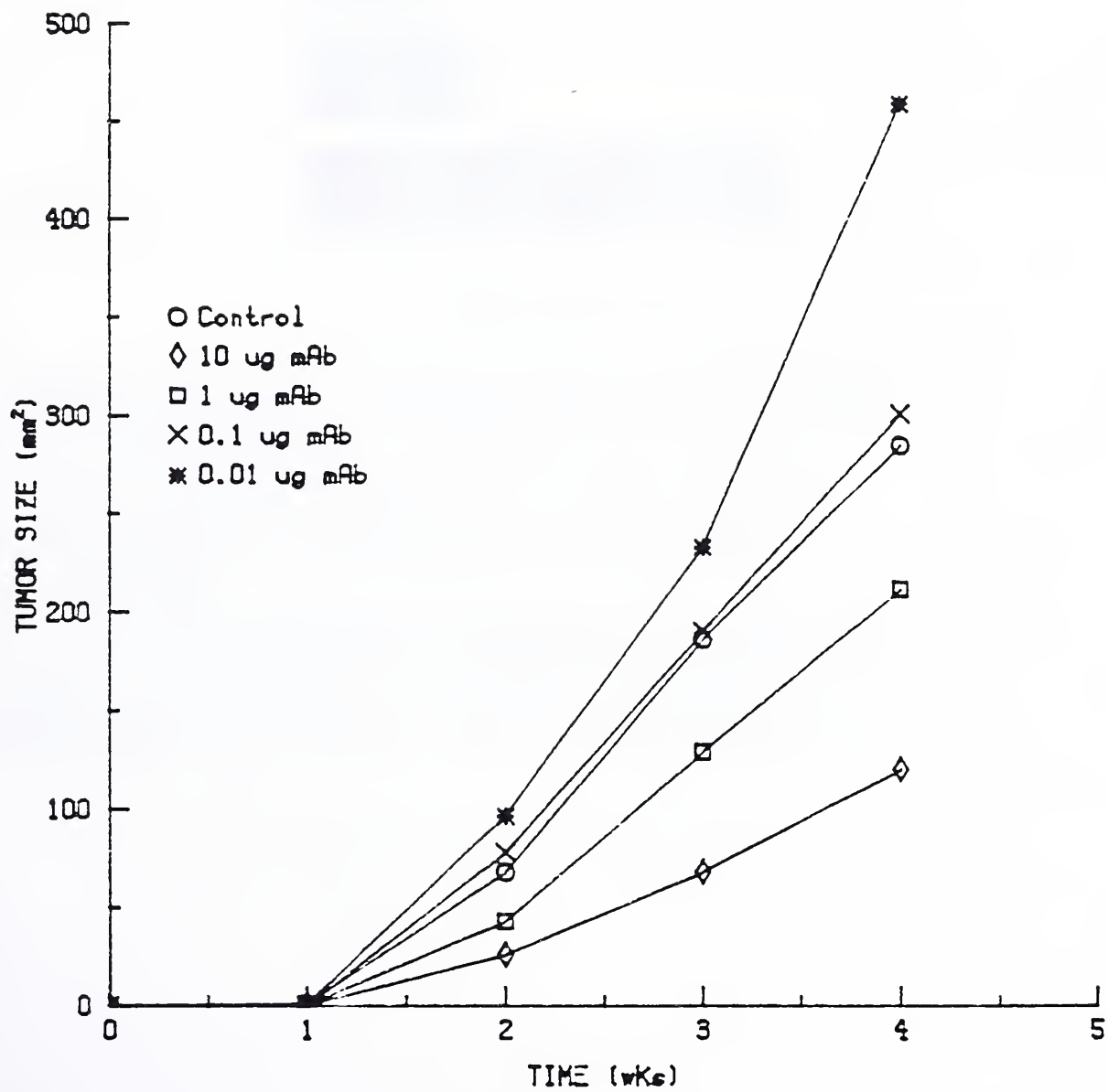




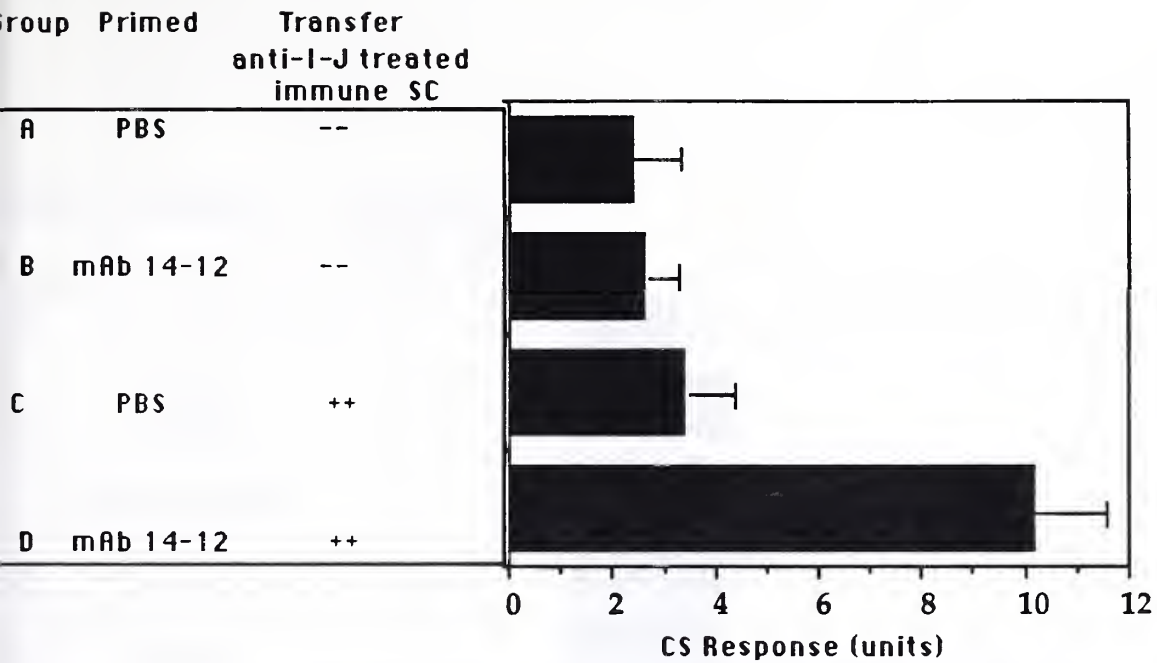


Figure 3.--The Effect of MAb 14-12 on the Growth of a Murine Fibrosarcoma is Dose-Related. Growth curves of the MCA-induced 31-52PRO tumor reveal that treatment with 10ug SF mAb 14-12 i.p. (3X/wk) was most effective and significantly inhibited tumor growth (wk 3 and 4  $p < 0.01$ ). Treatment with low doses (i.e. 0.1ug or less) resulted in augmentation of tumor growth. This augmentation effect was statistically significant by wk 4 ( $p < 0.01$ ) in animals receiving 0.01ug/inj. TBA/Challenged animals by week 4: 5/5 in all groups.

Mice were challenged with  $10^7$  cells 31-52PRO tumor cells s.q. on day 0 and tumor size was measured weekly by calculating tumor area. Tumor size for each group is expressed as mean tumor area of 5 mice. Statistical differences between experimental and control groups was established by student's t test.



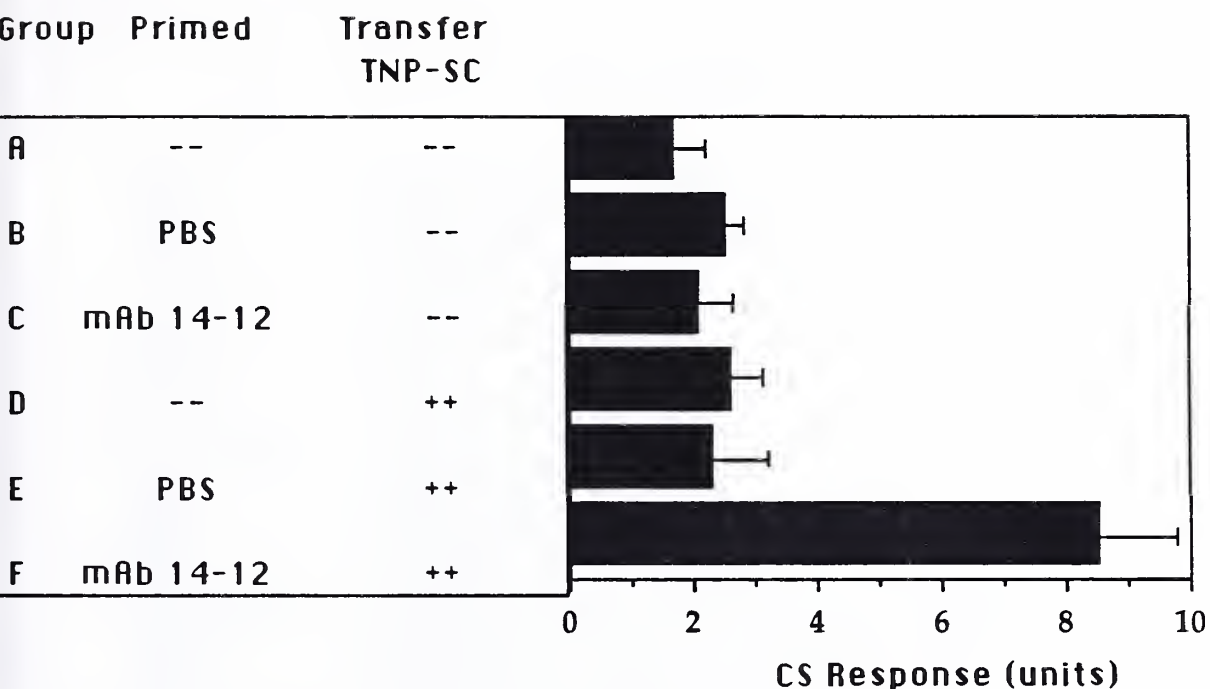




**Figure 4. --Treatment with MAb 14-12 Blocks the Suppression of the Adoptive Transfer of Contact Sensitivity to PCI.** CS responses to PCI in mice receiving 10ug SF mAb 14-12 (i.p.) 24 hours prior to transfer of anti-I-J -treated immune SC (Group D) were significantly different ( $p < 0.001$ ) from mice which received no immune cells (Groups A and B) or no mAb (Group C). Treatment with antibody alone (Group B) had no effect when compared to control animals (Group A).

All animals were challenged with PCI immediately after some groups (C and D) received  $3 \times 10^7$  immune spleen cells. CS responses are reported as the difference between the unchallenged left ear and the challenged right ear. 1 unit = 10-3cm. Error bars indicate S.E.M. Statistical difference was established by analysis with the student's t test. Each group consisted of 5 animals.





**Figure 5. --Treatment with MAb 14-12 Blocks Tolerance Induction to TNP and Converts a Tolerogenic Signal to an Immunogenic Signal.** Mice receiving 10ug SF mAb 14-12 (i.p.) 24 hours prior to transfer of a tolerogenic signal (TNP-SC i.v.; Group F) developed CS responses on subsequent challenge which were significantly different ( $p < 0.001$ ) from mice receiving no mAb (Groups D and E) or no cell transfer (Groups A, B, and C). Treatment with mAb alone (Group C) had no effect when compared to control animals (Group A).

All animals were challenged with PCI immediately after some groups (D,E, and F) received  $2 \times 10^7$  TNP-SC. CS responses are reported as the difference between the unchallenged left ear and the challenged right ear. 1 unit = 10-3cm. Error bars indicate S.E.M. Statistical difference was established by analysis with the student's t test. Each group consisted of 5 animals.



## LIST OF ABBREVIATIONS

APC	- antigen presenting cell
ATS	- anti-thymocyte serum
CMI	- cell-mediated immunity
CS	- contact sensitivity
ELISA	- enzyme-linked immunosorbent assay
FCS	- fetal calf serum
HBSS	- Hank's balanced salt solution
IL-2	- Interleukin-2
K cells	- killer cells
LAK cells	- Lymphokine-activated killer cells
mAb	- monoclonal antibody
MCA	- methylcholanthrene
MTV	- mammary tumor virus
NK cells	- natural killer cells
PAGE	- polyacrylamide gel electrophoresis
PBS	- phosphate-buffered saline
PCl	- picryl chloride
SAS	- saturated ammonium sulfate
SC	- spleen cells
SF	- serum-free
SPF	- specific pathogen free
SRBC	- sheep red blood cells
Tc cells	- cytotoxic T cells
Tcs cells	- contrasuppressor T cells
T <sub>DTH</sub> cell	- delayed-type hypersensitivity effector T cell
Th cell	- helper T cell
Ts cell	- suppressor T cell
TsF	- T cell suppressor factor
TAA	- tumor-associated antigen
TBA	- tumor-bearing animal
TNBS	- 2,4,6-Trinitrobenzene sulfonate
TNP	- trinitrophenol
TXB	- thymectomy, lethal radiation, bone marrow salvage
UV	- ultraviolet
VV+	- <u>Vicia villosa</u> adherent
VV-	- <u>Vicia villosa</u> nonadherent











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