Preface

The immune system plays a critical role in controlling and eliminating infectious organisms, including many pathogenic bacteria and viruses. More controversial has been the debate pertaining to whether the immune system can effectively control tumor growth and metastases. However, many studies suggest that appropriate activation of the immune system can lead to tumor regressions in experimental animal models. Thus, there is significant interest in harnessing the immune system for the treatment of tumors. The main focus of immunotherapy has been on T lymphocytes, since they have been shown to be the major effector cells in various animal tumor models. Removal of T cells typically eliminates the antitumor activity of most therapeutic approaches, while conversely, the adoptive transfer of tumor-reactive T cells mediates regression of malignant lesions. Furthermore, in several histologically distinct types of human tumors, the degree of T-cell infiltrate demonstrated a positive correlation with patient survival, suggesting a role for these cells in controlling malignant growth.

Significant progress has been made in the past several decades in our understanding of the host immune response to tumors. This has included: (1) identification of antigens expressed on human tumors as well as epitopes from these proteins that can serve as targets for the $CD4^+$ and $CD8^+$ T-cell populations; (2) defining and characterizing antigen presenting cells (e.g., dendritic cells), and the co-stimulatory requirements for effective peptide presentation; (3) identifying the role various cytokines play in regulating cellular and humoral immune responses; and (4) understanding the intracellular signaling pathways that control T and APC differentiation, effector functional and survival. There have also been important advances in our ability to monitor antitumor immune responses in tumor-bearing hosts. This has included the use of major histocompatibility complex (MHC)-tetramers to detect antigen-specific T cells in the blood and tumor, as well as the development of techniques to measure cytokine expression by subsets of T cells (ELISPOT, flow cytometry-based intracellular staining, and real-time PCR). These insights are leading to new approaches in immunotherapy, and to more precise ways of assessing the impact that such therapy has on antitumor effector T cells.

Prior clinical trials employing cytokines (IL-2 and IL-12) and interferons alone, or in different combinations, have demonstrated antitumor activity in select sets of patients. Overall, the response rate in patients with advanced disease has been in the 10–20% range. More recent clinical studies using various

vaccine strategies (peptides, peptide-pulsed dendritic cells, etc.) have demonstrated an ability to increase the frequency of tumor reactive T cells in the blood and in tumors. However, in the majority of these trials, the modest antitumor activity observed was not commensurate with the augmented number of effector cells. Although these studies suggest that boosting T cell-mediated antitumor immunity has some clinical activity, it currently is beneficial only to a minority of patients. It seems plausible that the effectiveness of immunotherapy will continue to improve as we develop more effective means of enhancing the appropriate effector cells through our better understanding of the tumor immune response at both the cellular and molecular levels. There is growing evidence, however, that tumors can evade the immune system by multiple mechanisms, each potentially representing a significant barrier to immunotherapy. Thus, understanding these processes may be critical to implementing new and more effective forms of immunotherapy.

It has been well documented that the tumor environment can have a negative impact on the development of an effective antitumor immune response. This concept is illustrated by the fact that a significant number of T cells infiltrating human tumors are functionally impaired in their ability to proliferate and mediate important effector functions. Furthermore, impaired immune function, including unresponsiveness to recall antigens, has been noted in peripheral blood T cells, suggesting that systemic effects can occur in cancer patients. There is also evidence to suggest that the antigen-specific T-cell response to some tumor antigens is impaired.

Part I of *Cancer Immunotherapy at the Crossroads: How Tumors Evade Immunity and What Can Be Done* outlines the basic mechanisms that may be operative in cancer patients that contribute to the poor development of antitumor immune responses. Tumors may escape detection by immune cells owing to defective MHC expression and/or antigen processing by the tumor, or because the tumors fail to migrate or interact with T cells at secondary lymphoid organs. Tumors may also evade the immune system by directly or indirectly modulating the normal activation and signaling cascades of immune cells. Indeed, tumors can alter the differentiation and function of dendritic cells, resulting in ineffective antigen presentation, and hence causing T-cell unresponsiveness or anergy. Thus, the tumor environment can impair both CD4+ helper and CD8+ effector T-cell responses. Also discussed within these chapters is the involvement of immunosuppressive products produced either by the tumor or the immune cells themselves, which are likely responsible for some of the immune dysfunction observed in both the antigen-presenting cells and T cells. It is also becoming clear that the tumor environment may alter the sensitivity of T cells and dendritic cells to programmed cell death, or apoptosis. This may occur as a natural response to antigen, leading to activation-induced cell death, or by the elaboration of tumor products that directly sensitize or induce apoptosis in immune cells.

Several chapters address mechanisms of optimizing antigen presentation and the delivery of T cells to tumor sites as well as ways to promote their survival. These modifications appear to enhance T-cell effector function and may render tumors less capable of immune evasion. Also discussed is the notion that malignant cells utilize some of the same immune escape mechanisms employed by various pathogens, suggesting that lessons learned from the study of infectious diseases may benefit the understanding of immune dysfunction in cancer. Although the majority of mechanisms examined in these pages focus on the tumor-induced dysfunction of immune cells, also included is a chapter appraising molecular alterations within the tumor cells themselves that afford resistance to apoptosis. These modifications enhance not only the resistance of tumors to immune-mediated attack, but also may significantly reduce their susceptibility to radiation and chemotherapy.

Additional chapters address immune dysfunction and evasion mechanisms in histologically diverse human tumors. These chapters highlight both the immunosuppressive tactics common to multiple tumor types, and the unique evasive mechanisms employed by biologically and histologically distinct tumors.

In Part II, the clinical relevance of immune evasion is reviewed. The functional and signaling defects in T cells and antigen-presenting cells and their relation to impaired antitumor immune responses and to poor clinical outcome are discussed. These investigations also ask whether measurably impaired signaling and effector function in T cells may one day serve as biomarkers for patient prognosis. These types of analysis are clearly important and suggest that defects in T cell signaling and immune function impact on clinical outcome, however, more studies are needed to address this issue.

The future development of effective immunotherapeutic protocols for treating cancer will incorporate strategies that can abrogate the mechanisms by which tumors evade the immune system in different histologic types of tumors. It is thus relevant to study and understand these evasion mechanisms in order to devise ways to prevent and/or circumvent their capacity to enhance progressive tumor growth.

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2 Immune Defects in T Cells From Cancer Patients

Parallels in Infectious Diseases

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CONTENTS

1. INTRODUCTION

The progressive growth of tumors in cancer and the chronic presence of a pathogenic microorganism reveal the inability of the immune response to eliminate the malignant cells and the chronic infectious agent. This setting is not

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uncommon in mice or patients with a congenital or acquired immunodeficiencies. However, this problem is also frequently seen in fully immunocompetent individuals, suggesting that malignant cells and certain microorganisms have developed mechanisms to evade the immune response. The distinct pathophysiology of cancer and chronic infections suggested that the mechanisms by which tumors and infectious agents impair and evade the immune response were different, and therefore, have been studied separately. Recent data however has demonstrated that T lymphocytes in cancer and chronic infections develop similar molecular alterations that lead to a state of unresponsiveness or anergy, suggesting the possibility that these seemingly disparate diseases may use similar pathways to impair the immune response. Here, we discuss some of the mechanisms that support this hypothesis and provide a possible common explanation to this phenomenon.

2. IMMUNE DYSFUNCTION IN CANCER

Seminal studies by Prehn and Main *(1,2)* demonstrated the existence of an immune response against tumor-specific antigens, raising the possibility that the immune response could be used to control tumor growth, and possibly to treat patients with cancer. Multiple studies in mice have confirmed the existence of a "surveillance mechanism" that can destroy tumor cells before they become an established malignancy of clinical significance *(3,4)*. However, studies in cancer patients failed to demonstrate a protective immune response, and instead suggested that the T-cell response was markedly impaired. In 1965, Hersh and Oppenheim *(5)* found that patients with Hodgkin's disease (HD) had a decreased delayed-type hypersensitivity (DTH) response to PPD and DNBC (di-nitrochlorobenzene) and a diminished in vitro response to mitogen stimulation. This immune dysfunction persisted, even in patients who had achieved a complete clinical response to chemotherapy *(6)*. Patients with melanoma also showed a decrease in the cellular immune response, but a marked increase in the levels of serum immunoglobulins. Similarly, patients with other solid tumors including renal cell carcinoma (RCC), prostate and bladder cancer *(7)*, lung cancer *(8)*, breast cancer *(9)*, and gastric cancer *(10)*, consistently showed a decreased cellular response. Several mechanisms were proposed to explain these observations. The high levels of antibodies observed in melanoma patients suggested the possibility that blocking antibodies might interfere with antigen recognition, preventing the priming and activation of T cells *(11,12)*. An alternative explanation suggested instead that the immune system was unable to recognize the original tumor inoculum because of its suboptimal antigenic load *(13)*. A third possibility came from cloning experiments that suggested the existence of T cells or macrophages with suppressor activity in the spleens of tumor-bearing mice *(14)*.

During the early 1980s, North and colleagues *(15–18)* demonstrated the presence of a protective antitumor T-cell response during the first days following tumor implantation, followed by a rapid decline with the appearance of $Ly1^+$ suppressor T cells by the second week. This suppressor function could be transferred into naive animals, and was eliminated with low doses of cyclophosphamide, re-establishing a therapeutic antitumor response. These findings provided insight into a dynamic interaction between tumors and the immune system, which could be manipulated to the benefit of the host. An alternative explanation came from studies on cytokine functions. Mossman and colleagues classified CD4+ T cells according to the cytokines produced. Th1 cells preferentially produced IL-2, IFN-γ, and TNF-α-promoting cellular responses, and Th2 cells secreted IL-4, IL-13, and IL-10, promoting antibody production *(19,20)*. Therefore, it was suggested that the progressive growth of tumor induced a loss of Th1 activity and an increased Th2 function, resulting in a diminished cellular response and an enhanced antibody production. Most of these concepts remained interesting research observations, but had minor relevance for the treatment of patients.

The advent of immunotherapy trials in the 1980s using the adoptive transfer of tumor-infiltrating lymphocytes (TIL) revealed to a greater extent the degree of T-cell dysfunction in patients with cancer. In vitro testing of freshly isolated TIL demonstrated that these cells had a markedly decreased proliferation when stimulated with mitogens or tumor cells, and had a significantly diminished clonogenic potential *(21–24)*. This cellular dysfunction appeared to have a detrimental effect on the therapeutic success of immunotherapy. Loeffler and colleagues *(25)* studied an immunotherapy model of adoptive transfer of T lymphocytes, demonstrating that T cells from mice bearing tumors for >21 d had a markedly diminished antitumor effect. In contrast, T cells from mice bearing tumors for <14 d had a high therapeutic efficacy when transferred into a tumor-bearing recipients. Further studies failed to demonstrate the presence of suppressor cells, yet they confirmed a significant decrease in T-cell cytotoxicity against tumor targets.

3. CHANGES IN T-CELL SIGNAL-TRANSDUCTION MOLECULES AND CANCER

In the mid-1980s, major advances in T-cell biology provided the basis for an understanding of the molecular events that lead to T-cell activation. Among these were the elucidation of the structure and function of T-cell receptor (TCR) and the mechanisms of T-cell signal transduction following antigen stimulation *(26–28)*. Briefly, two polymorphic chains, the α and β chains, confer antigen specificity to the T cell and form the antigen-binding site. These are covalently linked to the CD3 complex formed by the invariant chains γ, δ, ε, and ζ. The

latter forms ζζ homodimers (CD3ζ) or ζη heterodimers. Following TCR ligation, Src family tyrosine kinases, *p56*^{lck} (associated with CD4 or CD8) and *p59*^{fyn} phosphorylate CD3ζ and recruit ZAP-70, eventually leading to the activation of nuclear transcription factors such as NF-κB that translocate into the nucleus and activate or repress various genes, including cytokine genes *(29,30)*. Major advances have also been made in understanding the molecular changes that accompany T-cell unresponsiveness or anergy. Quill and colleagues *(31,32)* demonstrated that T cells stimulated by antigens presented on fixed antigen-presenting cells (APC) were anergic—e.g., unresponsive to repeated antigenic stimuli and unable to produce IL-2 *(33,34)*. Furthermore, stimulation of T cells with streptococcus superantigen produced a state of T-cell anergy and resulted in a decreased expression of *p56*^{lck} and *p59*^{fyn} *(31,35)*. Anergic T cells showed several molecular alterations, including the inability to phosphorylate *p21 ras (36,37)* and a decreased ability to activate nuclear transcription factors, including NF-κB and AP-1, important in regulating cytokine production *(38)*. These observations provided important tools to study the mechanisms for T-cell dysfunction in cancer.

Mizoguchi and colleagues *(39)* studied molecular changes in dysfunctional T cells from long-term tumor-bearing mice, and found alterations in the expression of several signal-transduction proteins, including a significant decrease in the expression of CD3ζ chain and *p56*^{lck} and *p59*^{fyn} tyrosine kinases. These changes were accompanied by a decreased tyrosine kinase phosphorylation and a diminished Ca^{++} flux. Li *(40)* and Ghosh *(41,42)* later showed that T cells from some patients with RCC and from long-term tumor-bearing mice were unable to translocate NF-κB*p65* nuclear transcription factor, which resulted in a predominance of NF-κB*p*50/50 homodimer in the nucleus, known to act as a repressor of the IFN-γ gene *(43)*. Indeed, cytokine production during the progressive growth of tumors in mice demonstrated a Th1 response (IL-2 and IFN-γ) early after tumor implantation, followed by an increased production of Th2 cytokines (IL-4 and IL-10) after the third week *(41,42)*. These findings provided for the first time a molecular basis to explain T-cell dysfunction in cancer.

Results in cancer patients confirmed the initial observations in tumor-bearing mice. T cells, and natural killer (NK) cells from approximately half of the patients with RCC, colon carcinoma, ovarian carcinoma, gastric cancer, breast cancer, prostate cancer, Hodgkin's disease, acute myelocytic leukemia, and other tumors showed a decreased expression of the CD3ζ chain and a decreased in vitro response to antigens or mitogens *(44–49)*. Alterations in signal-transduction molecules were most markedly observed in T cells that infiltrated tumors or T cells from lymph nodes draining the site of the tumor. In addition, T cells from RCC patients exhibited a diminished ability to translocate NF-κB*p65*. However, changes in signal-transduction molecules were not limited to those associated with the TCR. Kolenko and colleagues demonstrated

that Jak-3, a tyrosine kinase associated with the γ chain, a common element to IL-2, IL-4, IL-7, and IL-15 cytokine receptors, was also decreased in T cells from RCC patients *(50)*.

Controversy over these findings arose when Levey and Srivastava *(51)* had difficulty reproducing some of the original observations in mice with transplantable tumors. However, Horiguchi and colleagues *(52)* later demonstrated that de novo tumors (chemically induced) were able to induce all of the alterations in T-cell signal-transduction molecules described initially, yet after repeated passage in mice, the tumors failed to cause T-cell alterations. Thus, it was possible that the tumors used by Levey and Srivastava may have lost their ability to induce immune dysfunction after multiple passages.

Initial work in colon carcinoma *(53)* and RCC *(54)* suggested that patients with more advanced stages of the disease had a higher frequency of T-cell signal-transduction alterations. In addition, TIL in RCC and ovarian carcinoma, as well as T cells from draining lymph nodes, had a more pronounced decrease in the expression of signal-transduction proteins than peripheral-blood T cells. However, in cervical carcinoma *(55)*, some patients with carcinoma *in situ*, an early stage of the disease, already showed a diminished expression of CD3ζ, suggesting that T-cell signal-transduction alterations were not an exclusive characteristic of the advanced stages of cancer. Other reports have also suggested an association between the expression of CD3ζ and survival (*see* Chapter 14). Patients with metastatic melanoma (Stage IV) and patients with head and neck cancer who had normal levels of CD3ζ chain had a significantly longer survival as compared to those who had undetectable levels of the same proteins *(57,58)*.

The expression of CD3ζ changes with treatment. Patients with non-Hodgkin's lymphoma (NHL) and patients with HD *(59,60)* who responded to chemotherapy showed a re-expression of normal levels of ζ chain, which decreased again in patients who had a recurrence of the disease *(61)*. Patients who received immunotherapy were also tested for possible changes in the expression of CD3ζ and other signal-transduction proteins. Farace and colleagues *(62)* initially reported that the decreased expression of CD3ζ chain was not corrected by the infusion of IL-2 alone. In contrast, data from several groups with ovarian carcinoma, melanoma, RCC, and colon carcinoma showed that patients who received IL-2 based therapies had a recovery of the levels of CD3ζ *(47,54)*. It is possible that these contrasting results could be explained by the different doses of IL-2 being infused. Re-expression of normal levels of ζ chain did not always coincide with a full recovery of T-cell function. Tyrosine kinase activity, tyrosine kinase phosphorylation patterns, and the production of cytokines were not always fully restored, suggesting that the expression of tyrosine kinases and/or nuclear transcription factors might not be normalized by IL-2 alone. More recently, Finn and colleagues *(63)* found that patients who

received a MUC-1 vaccine and developed a positive DTH to the vaccine recovered the expression of CD3ζ. In a retrospective study, Gratama et al. *(64)* found that RCC patients that achieved a complete response when treated with IL-2 + IFN-α and LAK cells had a complete recovery of both CD3ζ chain and $p56^{lck}$ tyrosine kinase expression. In contrast, <25% of those with progressive disease had a partial re-expression of CD3ζ, and none recovered $p56^{lck}$. These data, although preliminary, suggest that monitoring the expression of signal-transduction proteins may provide a method for evaluating the response to immunotherapy, and could provide prognostic information.

4. MECHANISMS LEADING TO ALTERATIONS IN T-CELL SIGNAL TRANSDUCTION IN CANCER

Most of the studies on mechanisms that cause alterations in T-cell signal transduction have been conducted in murine tumor models. In a series of elegant in vitro experiments, Otsuji et al. *(65)* and Kono et al. *(55,66)* demonstrated that H_2O_2 from macrophages induced the loss of CD3ζ chain in naive T cells, a phenomenon that could be blocked by the depletion of macrophages or the addition of oxygen radical scavengers *(67)*. These observations were extended in patients by Schmielau et al. *(68)*, who suggested that H_2O_2 from neutrophils could induce similar alterations. A second mechanism leading to loss of the CD3ζ chain was found while studying Fas-FasL-induced T-cell apoptosis *(69–73)*. When T cells undergo apoptosis, they lose the expression of CD3ζ as one of the early changes in this process. Therefore, the diminished expression of the CD3ζ chain seen in cancer patients could be partly explained by an increased frequency of apoptotic cells in peripheral blood. However, these mechanisms have not yet been studied in infectious diseases.

5. T-CELL DYSFUNCTION IN CHRONIC INFECTIONS: LESSONS FROM OTHER DISEASES

The development of mechanisms to disrupt the immune response has been an active field of study in chronic infectious diseases. The chronicity of certain infections demonstrates the inability of the immune response alone to eliminate the infectious agent, and therefore suggests that the microorganisms have developed ways of blocking a protective immune response. In some infections, this process is represented by different clinical forms of the disease. Mycobacterial diseases, particularly leprosy, have provided a model for the study of changes in the immune response in chronic infections with different clinical presentations. Leprosy has two major polar forms that are closely associated with differences in the immune response. The tuberculoid form is clinically characterized by the presence of anesthetic and discolored patches of skin,

Table 1
Changes in Signal Transduction in Cancer and Mycobacterial Infections

Changes in Signal Transduction	*Cancer*	*Mycobacterial Infections*
Decreased CD3ζ chain expression	+++	+++
Decreased $p65^{lck}$	+++	+++
Decreased $p59^{fyn}$	+++	?
Inability to translocate NF-κB*p65*	+++	+++
Decreased Ca^{++} flux	++	?

a limited number of bacteria, and the presence of an active T-cell response to lepromin. At the other end of the spectrum are patients with lepromatous leprosy, who have significantly more advanced tissue damage and a large bacterial burden, and have lost the DTH to lepromin antigens. Various studies have demonstrated a predominance of Th_1 response with the production of IFN-γ and TNF-α in tuberculoid leprosy and a predominance of Th2 cytokines in lepromatous leprosy. The injection of IL-2 in the lepromatous nodules reverses this polarization of the immune response and confers an anti-mycobacterial effect *(74)*. Therefore, it has been suggested that the predominance of a Th2 response could provide a partial explanation for the lack of a protective response in lepromatous leprosy. However, despite a strong Th1 response in tuberculoid leprosy, patients still develop the disease, suggesting that other immune alterations might play a role in its pathogenesis. Zea et al. *(56)* studied T-cell signal transduction in these patients and found that, as in cancer patients, patients with lepromatous leprosy had a decreased expression of CD3ζ chain and $p56^{lck}$ tyrosine kinase and were unable to translocate NF-κB*p65*. In addition, some of the patients with tuberculoid leprosy also had a decreased expression of $p56^{lck}$, although less frequently than lepromatous patients (Table 1). Similar observations have been made in tuberculosis *(75)* and other non-mycobacterial infections such as *Helicobacter pylori* (Zabaleta, J., et al., submitted).

6. MODULATION OF T-CELL FUNCTION AND CD3ζ EXPRESSION BY ARGININE AVAILABILITY: A COMMON LINK BETWEEN CANCER AND INFECTIOUS DISEASES?

L-arginine is a non-essential amino acid that plays a central role in various biological systems, including the immune response. Taheri et al. and Rodriguez and colleagues *(76,77)*, recently demonstrated that T cells cultured in the absence of arginine lose the expression of CD3ζ, and exhibit a decreased pro-

liferation and a diminished production of IFN-γ. In vivo levels of arginine are maintained by dietary intake and endogenous synthesis, and are decreased by catabolism of the amino acid through three enzymatic pathways, nitric oxide synthase (NOS), and arginase I and II. NOS uses arginine as the substrate to produce nitric oxide (NO), a major cytotoxic mechanism in macrophages and an important mediator of vascular homeostasis. Arginase I (cytoplasmic form) and II (mitochondrial form) use arginine to produce polyamines that are essential for cell proliferation and urea as a method of detoxification. In macrophages, IFN-γ upregulates iNOS, and Th2 cytokines IL-4 and IL-13 upregulate arginase I. Rodriguez et al. *(78)* recently showed that arginase I rapidly depletes L-arginine from the microenvironment, causing the loss of CD3ζ, and resulting in profound T-cell dysfunction. Arginase is produced by macrophages as well as tumor cells that may further enhance the depletion of arginine. Therefore, T cells that infiltrate the tumor and recognize tumor antigens could undergo the loss of CD3ζ chain and other signal-transduction proteins as a result of the arginine depletion, which would effectively prevent the development of an antitumor response.

Arginase is also produced by microorganisms as a means of synthesizing polyamines or other proteins needed for their survival. Some of the microorganisms that produce arginase are also the cause of chronic human disease, including *Helicobacter pylori* and leishmaniasis *(79–82)*. In *H. pylori*, for example, arginase is used to produce urea, which in turn serves as the substrate for the enzyme urease that produces the ammonia needed to neutralize the acidity of the stomach where the bacteria lives. Other examples include leprosy, in which an increased arginase activity has been described in the serum of lepromatous patients *(83)*. However, it is unclear from these reports whether arginase is produced by the microorganism or by infected monocytes. Results from patients with tuberculosis suggest that the increased production of arginase in mycobacterial infections may instead come from arginase produced by macrophages (A. Zea, submitted manuscript). Therefore, arginase produced by microorganisms or by the immune cells of infected patients could provide an important means of evading the immune response through similar mechanisms to tumors.

7. CONCLUSIONS

Tumors and chronic infections induce similar molecular alterations in T cells (Table 1), including the loss of CD3ζ chain, a decreased expression of $p56^{lck}$ tyrosine kinase, and the inability to translocate NF-κB*p65*, which lead to a dysfunctional T-cell response and suggest a common mechanism (Fig. 1). Therefore, we have proposed that arginase produced by tumor cells, macrophages, or microorganisms could deplete arginine in tissues, causing the

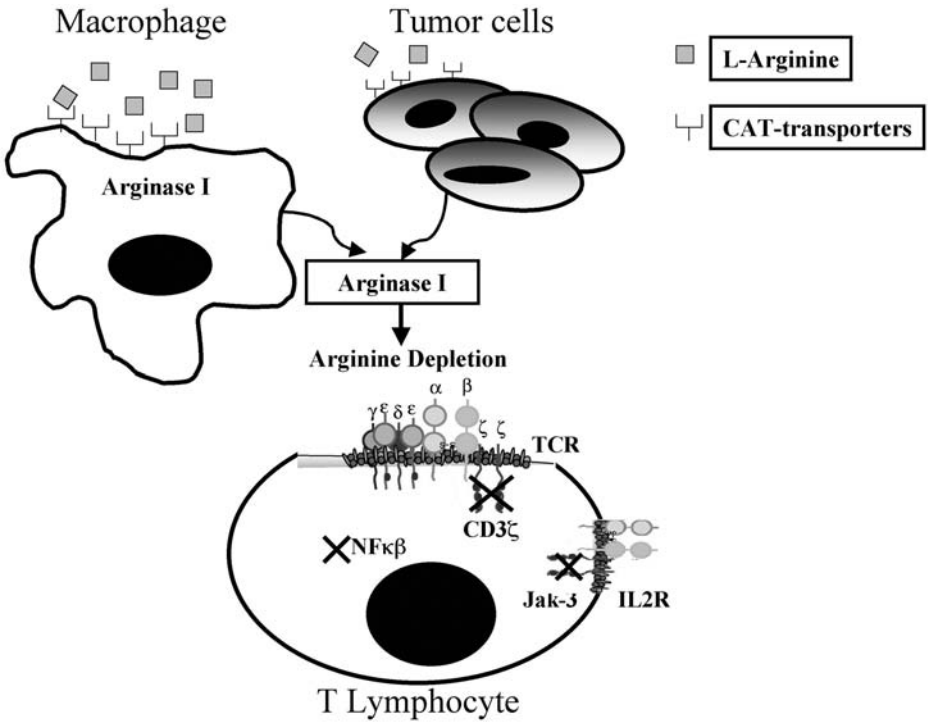

Fig. 1. Arginase produced by macrophages, tumor cells, or microorganisms depletes arginine from the microenvironment and causes alterations in T-cell signal transduction, which results in a state of anergy to the specific antigens.

loss of CD3ζ and inducing a state of anergy in the T cells infiltrating the site. Alternatively, if enough arginase is produced systemically as a result of metastatic tumor or a chronic and disseminated infection (as in miliary tuberculosis), the depletion of arginine could affect the function of specific T cells as well as those that recognize unrelated antigens. Therefore, despite the different pathophysiology of these diseases, a common mechanism leading to these changes appears to be the regulation of arginine availability. Further studies will be required to determine the impact of these changes in the outcome of the disease and the potential for the development of therapies that may prevent and/or reverse the development of T-cell alterations and T-cell anergy.

REFERENCES

1. Pliskin ME, Prehn RT. Are tumor-associated transplantation antigens of chemically induced sarcomas related to alien histocompatibility antigens? *Transplantation* 1978;26:19–24.
2. Prehn RT, Main JM. Immunity to methylcholanthrene-induced sarcomas. *J Natl Cancer Inst* 1957; 18:759–778.

3. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. *Nat Immunol* 2002;3:991–998.
4. Urban JL, Schreiber H. Host-tumor interactions in immunosurveillance against cancer. *Prog Exp Tumor Res* 1988;32:17–68.
5. Hersh EM, Oppenheim JJ. Impaired in vitro lymphocyte transformation in Hodgkin's disease. *N Engl J Med* 1965;273:1006–1012.
6. Fisher RI, DeVita VT, Jr., Bostick F, Vanhaelen C, Howser DM, Hubbard SM, et al. Persistent immunologic abnormalities in long-term survivors of advanced Hodgkin's disease. *Ann Intern Med* 1980;92:595–599.
7. Catalona WJ, Smolev JK, Harty JI. Prognostic value of host immunocompetence in urologic cancer patients. *J Urol* 1975;114:922–926.
8. Alberola V, Gonzalez-Molina A, Trenor A, San Martin B, Lluch A, Palau F, et al. Mechanism of suppression of the depressed lymphocyte response in lung cancer patients. *Allergol Immunopathol (Madr)* 1985;13:213–219.
9. Jerrells TR, Dean JH, Richardson GL, McCoy JL, Herberman RB. Role of suppressor cells in depression of in vitro lymphoproliferative responses of lung cancer and breast cancer patients. *J Natl Cancer Inst* 1978;61:1001–1009.
10. Iwahashi M, Tanimura H, Yamaue H, Tsunoda T, Tani M, Tamai M, et al. Defective autologous mixed lymphocyte reaction (AMLR) and killer activity generated in the AMLR in cancer patients. *Int J Cancer* 1992;51:67–71.
11. Hellstrom I, Sjogren HO, Warner G, Hellstrom KE. Blocking of cell-mediated tumor immunity by sera from patients with growing neoplasms. *Int J Cancer* 1971;7:226–237.
12. Hellstrom KE, Hellstrom I, Nelson K. Antigen-specific suppressor ("blocking") factors in tumor immunity. *Biomembranes* 1983;11:365–388.
13. Mengersen R, Schick R, Kolsch E. Correlation of "sneaking through" of tumor cells with specific immunological impairment of the host. *Eur J Immunol* 1975;5:532–537.
14. Varesio L, Giovarelli M, Landolfo S, Forni G. Suppression of proliferative response and lymphokine production during the progression of a spontaneous tumor. *Cancer Res* 1979;39:4983–4988.
15. Dye ES, North RJ. Specificity of the T cells that mediate and suppress adoptive immunotherapy of established tumors. *J Leukoc Biol* 1984;36:27–37.
16. Mills CD, North RJ. Ly-1+2- suppressor T cells inhibit the expression of passively transferred antitumor immunity by suppressing the generation of cytolytic T cells. *Transplantation* 1985;39:202–208.
17. North RJ, Bursuker I. Generation and decay of the immune response to a progressive fibrosarcoma. I. Ly-1+2- suppressor T cells down-regulate the generation of Ly-1-2+ effector T cells. *J Exp Med* 1984;159:1295–1311.
18. North RJ. Down-regulation of the antitumor immune response. *Adv Cancer Res* 1985;45:1–43.
19. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989;7:145–173.
20. Mosmann TR, Schumacher JH, Street NF, Budd R, O'Garra A, Fong TA, et al. Diversity of cytokine synthesis and function of mouse CD4+ T cells. *Immunol Rev* 1991;123: 209–229.
21. Miescher S, Whiteside TL, Carrel S, von F, V. Functional properties of tumor-infiltrating and blood lymphocytes in patients with solid tumors: effects of tumor cells and their supernatants on proliferative responses of lymphocytes. *J Immunol* 1986;136:1899–1907.
22. Miescher S, Stoeck M, Qiao L, Barras C, Barrelet L, von F, V. Preferential clonogenic deficit of CD8-positive T-lymphocytes infiltrating human solid tumors. *Cancer Res* 1988; 48:6992–6998.

23. Whiteside TL, Miescher S, Moretta L, von F, V. Cloning and proliferating precursor frequencies of tumor-infiltrating lymphocytes from human solid tumors. *Transplant Proc* 1988;20:342–343.
24. Whiteside TL, Rabinowich H. The role of Fas/FasL in immunosuppression induced by human tumors. *Cancer Immunol Immunother* 1998;46:175–184.
25. Loeffler CM. Antitumor effects of interleukin 2 liposomes and anti-CD3-stimulated T-cells against murine MCA-38 hepatic metastasis. *Cancer Res* 1991;51:2127–2132.
26. Frank SJ, Samelson LE, Klausner RD. The structure and signalling functions of the invariant T cell receptor components. *Semin Immunol* 1990;2:89–97.
27. Weiss A, Desai D, Graber M, Picus J, Koretzky G. The regulation of T-cell antigen receptor signal transduction function. *Transplant Proc* 1991;23:32–33.
28. Weissman AM, Baniyash M, Hou D, Samelson LE, Burgess WH, Klausner RD. Molecular cloning of the zeta chain of the T cell antigen receptor. *Science* 1988;239:1018–1021.
29. Bockenstedt LK, Goldsmith MA, Koretzky GA, Weiss A. The activation of T lymphocytes. *Rheum Dis Clin North Am* 1987;13:411–430.
30. Fraser JD, Straus D, Weiss A. Signal transduction events leading to T-cell lymphokine gene expression. *Immunol Today* 1993;14:357–362.
31. Quill H, Schwartz RH. Stimulation of normal inducer T cell clones with antigen presented by purified Ia molecules in planar lipid membranes: specific induction of a long-lived state of proliferative nonresponsiveness. *J Immunol* 1987;138:3704–3712.
32. Quill H, Riley MP, Cho EA, Casnellie JE, Reed JC, Torigoe T. Anergic Th1 cells express altered levels of the protein tyrosine kinases p56lck and p59fyn. *J Immunol* 1992;149:2887–2893.
33. DeSilva DR, Urdahl KB, Jenkins MK. Clonal anergy is induced in vitro by T cell receptor occupancy in the absence of proliferation. *J Immunol* 1991;147:3261–3267.
34. Jenkins MK, Schwartz RH. Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness in vitro and in vivo. *J Exp Med* 1987;165:302–319.
35. Cho EA, Riley MP, Sillman AL, Quill H. Altered protein tyrosine phosphorylation in anergic Th1 cells. *J Immunol* 1993;151:20–28.
36. Fields PE, Gajewski TF, Fitch FW. Blocked Ras activation in anergic CD4+ T cells. *Science* 1996;271:1276–1278.
37. Gajewski TF, Qian D, Fields P, Fitch FW. Anergic T-lymphocyte clones have altered inositol phosphate, calcium, and tyrosine kinase signaling pathways. *Proc Natl Acad Sci USA* 1994;91:38–42.
38. Mondino A, Whaley CD, DeSilva DR, Li W, Jenkins MK, Mueller DL. Defective transcription of the IL-2 gene is associated with impaired expression of c-Fos, FosB, and JunB in anergic T helper 1 cells. *J Immunol* 1996;157:2048–2057.
39. Mizoguchi H, O'Shea JJ, Longo DL, Loeffler CM, McVicar DW, Ochoa AC. Alterations in signal transduction molecules in T lymphocytes from tumor-bearing mice. *Science* 1992;258:1795–1798.
40. Li X, Liu J, Park JK, Hamilton TA, Rayman P, Klein E, et al. T cells from renal cell carcinoma patients exhibit an abnormal pattern of kappa B-specific DNA-binding activity: a preliminary report. *Cancer Res* 1994;54:5424–5429.
41. Ghosh P, Komschlies KL, Cippitelli M, Longo DL, Subleski J, Ye J, et al. Gradual loss of T-helper 1 populations in spleen of mice during progressive tumor growth. *J Natl Cancer Inst* 1995;87:1478–1483.
42. Ghosh P, Sica A, Young HA, Ye J, Franco JL, Wiltrout RH, et al. Alterations in NF kappa B/Rel family proteins in splenic T-cells from tumor-bearing mice and reversal following therapy. *Cancer Res* 1994;54:2969–2972.

43. Young HA, Ghosh P. Molecular regulation of cytokine gene expression: interferon-gamma as a model system. *Prog Nucleic Acid Res Mol Biol* 1997;56:109–127.
44. Buggins AG, Hirst WJ, Pagliuca A, Mufti GJ. Variable expression of CD3-zeta and associated protein tyrosine kinases in lymphocytes from patients with myeloid malignancies. *Br J Haematol* 1998;100:784–792.
45. Finke JH, Zea AH, Stanley J, Longo DL, Mizoguchi H, Tubbs RR, et al. Loss of T-cell receptor zeta chain and p56lck in T-cells infiltrating human renal cell carcinoma. *Cancer Res* 1993;53:5613–5616.
46. Gunji Y, Hori S, Aoe T, Asano T, Ochiai T, Isono K, et al. High frequency of cancer patients with abnormal assembly of the T cell receptor-CD3 complex in peripheral blood T lymphocytes. *Jpn J Cancer Res* 1994;85:1189–1192.
47. Rabinowich H, Suminami Y, Reichert TE, Crowley-Nowick P, Bell M, Edwards R, et al. Expression of cytokine genes or proteins and signaling molecules in lymphocytes associated with human ovarian carcinoma. *Int J Cancer* 1996;68:276–284.
48. Rossi E, Matutes E, Morilla R, Owusu-Ankomah K, Heffernan AM, Catovsky D. Zeta chain and CD28 are poorly expressed on T lymphocytes from chronic lymphocytic leukemia. *Leukemia* 1996;10:494–497.
49. Ungefroren H, Voss M, Bernstorff WV, Schmid A, Kremer B, Kalthoff H. Immunological escape mechanisms in pancreatic carcinoma. *Ann NY Acad Sci* 1999;880:243–251.
50. Kolenko V, Wang Q, Riedy MC, O'Shea J, Ritz J, Cathcart MK, et al. Tumor-induced suppression of T lymphocyte proliferation coincides with inhibition of Jak3 expression and IL-2 receptor signaling: role of soluble products from human renal cell carcinomas. *J Immunol* 1997;159:3057–3067.
51. Levey DL, Srivastava PK. T cells from late tumor-bearing mice express normal levels of p56lck, p59fyn, ZAP-70, and CD3 zeta despite suppressed cytolytic activity. *J Exp Med* 1995;182:1029–1036.
52. Horiguchi S, Petersson M, Nakazawa T, Kanda M, Zea AH, Ochoa AC, et al. Primary chemically induced tumors induce profound immunosuppression concomitant with apoptosis and alterations in signal transduction in T cells and NK cells. *Cancer Res* 1999;59:2950–2956.
53. Nakagomi H, Petersson M, Magnusson I, Juhlin C, Matsuda M, Mellstedt H, et al. Decreased expression of the signal-transducing zeta chains in tumor-infiltrating T-cells and NK cells of patients with colorectal carcinoma. *Cancer Res* 1993;53:5610–5612.
54. Bukowski RM, Rayman P, Uzzo R, Bloom T, Sandstrom K, Peereboom D, et al. Signal transduction abnormalities in T lymphocytes from patients with advanced renal carcinoma: clinical relevance and effects of cytokine therapy. *Clin Cancer Res* 1998;4:2337–2347.
55. Kono K, Ressing ME, Brandt RM, Melief CJ, Potkul RK, Andersson B, et al. Decreased expression of signal-transducing zeta chain in peripheral T cells and natural killer cells in patients with cervical cancer. *Clin Cancer Res* 1996;2:1825–1828.
56. Zea AH, Ochoa MT, Ghosh P, Longo DL, Alvord WG, Valderrama L, et al. Changes in expression of signal transduction proteins in T lymphocytes of patients with leprosy. *Infect Immun* 1998;66:499–504.
57. Zea AH, Corti BD, Longo DL, Alvord WG, Strobl SL, Mizoguchi H, et al. Alterations in T Cell receptor and signal transduction molecules in melanoma patients. *Clin Cancer Res* 1995;1:1327–1335.
58. Reichert TE, Day R, Wagner EM, Whiteside TL. Absent or low expression of the zeta chain in T cells at the tumor site correlates with poor survival in patients with oral carcinoma. *Cancer Res* 1998;58:5344–5347.
59. Massaia M, Attisano C, Beggiato E, Bianchi A, Pileri A. Correlation between disease activity and T-cell CD3 zeta chain expression in a B-cell lymphoma. *Br J Haematol* 1994;88:886–888.

60. Renner C, Ohnesorge S, Held G, Bauer S, Jung W, Pfitzenmeier JP, et al. T cells from patients with Hodgkin's disease have a defective T-cell receptor zeta chain expression that is reversible by T-cell stimulation with CD3 and CD28. *Blood* 1996;88:236–241.
61. Massaia M, Attisano C, Beggiato E, Bianchi A, Pileri A. Correlation between disease activity and T-cell CD3 zeta chain expression in a B-cell lymphoma. *Br J Haematol* 1994;88:886–888.
62. Farace F, Angevin E, Vanderplancke J, Escudier B, Triebel F. The decreased expression of CD3 zeta chains in cancer patients is not reversed by IL-2 administration. *Int J Cancer* 1994;59:752–755.
63. Finn OJ, Lotze MT. A decade in the life of tumor immunology. *Clin Cancer Res* 2001; 7:759s–760s.
64. Gratama JW, Zea AH, Bolhuis RL, Ochoa AC. Restoration of expression of signal-transduction molecules in lymphocytes from patients with metastatic renal cell cancer after combination immunotherapy. *Cancer Immunol Immunother* 1999;48:263–269.
65. Otsuji M, Kimura Y, Aoe T, Okamoto Y, Saito T. Oxidative stress by tumor-derived macrophages suppresses the expression of CD3 zeta chain of T-cell receptor complex and antigen-specific T-cell responses. *Proc Natl Acad Sci USA* 1996;93:13,119–13,124.
66. Kono K, Salazar-Onfray F, Petersson M, Hansson J, Masucci G, Wasserman K, et al. Hydrogen peroxide secreted by tumor-derived macrophages down-modulates signal-transducing zeta molecules and inhibits tumor-specific T cell-and natural killer cell-mediated cytotoxicity. *Eur J Immunol* 1996;26:1308–1313.
67. Corsi MM, Maes HH, Wasserman K, Fulgenzi A, Gaja G, Ferrero ME. Protection by L-2-oxothiazolidine-4-carboxylic acid of hydrogen peroxide-induced CD3zeta and CD16zeta chain down-regulation in human peripheral blood lymphocytes and lymphokine-activated killer cells. *Biochem Pharmacol* 1998;56:657–662.
68. Schmielau J, Finn OJ. Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of t-cell function in advanced cancer patients. *Cancer Res* 2001;61:4756–4760.
69. Gastman BR, Johnson DE, Whiteside TL, Rabinowich H. Caspase-mediated degradation of T-cell receptor zeta-chain. *Cancer Res* 1999;59:1422–1427.
70. Rabinowich H, Reichert TE, Kashii Y, Gastman BR, Bell MC, Whiteside TL. Lymphocyte apoptosis induced by Fas li. *J Clin Invest* 1998;101:2579–2588.
71. Uzzo RG, Rayman P, Kolenko V, Clark PE, Cathcart MK, Bloom T, et al. Renal cell carcinoma-derived gangliosides suppress nuclear factor-kappaB activation in T cells. *J Clin Investig* 1999;104:769–776.
72. Uzzo RG, Rayman P, Kolenko V, Clark PE, Bloom T, Ward AM, et al. Mechanisms of apoptosis in T cells from patients with renal cell carcinoma. *Clin Cancer Res* 1999;5: 1219–1229.
73. Uzzo RG, Clark PE, Rayman P, Bloom T, Rybicki L, Novick AC, et al. Alterations in NFkappaB activation in T lymphocytes of patients with renal cell carcinoma. *J Natl Cancer Inst* 1999;91:718–721.
74. Kaplan G, Kiessling R, Teklemariam S, Hancock G, Sheftel, Job CK, et al. The reconstitution of cell-mediated immunity in the cutaneous lesions of lepromatous leprosy by recombinant interleukin 2. *J Exp Med* 1989;169:893–907.
75. Zea AH, Culotta K, Ali J, Mason C, Hae-Joon P, Zabaleta J, et al. Alterations in T cell signal transduction proteins in patients with tuberculosis. *FASEB J* 2002; *Exp Biol* 2002;A300.
76. Rodriguez PC, Zea AH, Culotta KS, Zabaleta J, Ochoa JB, Ochoa AC. Regulation of T cell receptor CD3 zeta chain expression by L-arginine. *J Biol Chem* 2002;277:21,123–21,129.
77. Taheri F, Ochoa JB, Faghiri Z, Culotta K, Park HJ, Lan MS, et al. L-Arginine regulates the expression of the T-cell receptor zeta chain (CD3zeta) in Jurkat cells. *Clin Cancer Res* 2001;7:958s–965s.

78. Rodriguez PC, Zea AH, DeSalvo J, Culotta KS, Zabuleta J, Quiceno DG, et al. L-Arginine consumption by macrophages modulates the expression of CD3ζ chain in T lymphocytes. *J Immunol* 2003;17:1232–1239.
79. da Silva ER, Castilho TM, Pioker FC, Tomich de Paula Silva CH, Floeter-Winter LM. Genomic organisation and transcription characterisation of the gene encoding Leishmania (Leishmania) amazonensis arginase and its protein structure prediction. *Int J Parasitol* 2002;32:727–737.
80. Iniesta V, Gomez-Nieto LC, Corraliza I. The inhibition of arginase by N(omega)-hydroxy-l-arginine controls the growth of Leishmania inside macrophages. *J Exp Med* 2001;193:777–784.
81. Iniesta V, Gomez-Nieto LC, Molano I, Mohedano A, Carcelen J, Miron C, et al. Arginase I induction in macrophages, triggered by Th2-type cytokines, supports the growth of intracellular Leishmania parasites. *Parasite Immunol* 2002;24:113–118.
82. Mendz GL, Holmes EM, Ferrero RL. In situ characterization of Helicobacter pylori arginase. *Biochim Biophys Acta* 1998;1388:465–477.
83. Babu CS, Kannan KB, Bharadwaj VP, Katoch VM. Lymphocyte arginase activity in leprosy—a preliminary report. *Indian J Med Res* 1990;91:193–196.