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Immune Defects in T Cells From Cancer Patients

Parallels in Infectious Diseases

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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 $\zeta\zeta$ homodimers (CD3 ζ) or $\zeta\eta$ heterodimers. Following TCR ligation, Src family tyrosine kinases, $p56^{\text{lck}}$ (associated with CD4 or CD8) and $p59^{\text{fyn}}$ phosphorylate CD3 ζ and recruit ZAP-70, eventually leading to the activation of nuclear transcription factors such as NF- κ B 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 $p56^{\text{lck}}$ and $p59^{\text{fyn}}$ (31,35). Anergic T cells showed several molecular alterations, including the inability to phosphorylate $p21 \text{ ras}$ (36,37) and a decreased ability to activate nuclear transcription factors, including NF- κ B 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 CD3 ζ chain and $p56^{\text{lck}}$ and $p59^{\text{fyn}}$ 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- κ Bp65 nuclear transcription factor, which resulted in a predominance of NF- κ Bp50/50 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 CD3 ζ 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- κ Bp65. 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 CD3 ζ , 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 CD3 ζ and survival (*see* Chapter 14). Patients with metastatic melanoma (Stage IV) and patients with head and neck cancer who had normal levels of CD3 ζ chain had a significantly longer survival as compared to those who had undetectable levels of the same proteins (57,58).

The expression of CD3 ζ 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 CD3 ζ and other signal-transduction proteins. Farace and colleagues (62) initially reported that the decreased expression of CD3 ζ 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 CD3 ζ (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 CD3 ζ . 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 CD3 ζ chain and p56^{lck} tyrosine kinase expression. In contrast, <25% of those with progressive disease had a partial re-expression of CD3 ζ , 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 H₂O₂ from macrophages induced the loss of CD3 ζ 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 H₂O₂ from neutrophils could induce similar alterations. A second mechanism leading to loss of the CD3 ζ chain was found while studying Fas-FasL-induced T-cell apoptosis (69–73). When T cells undergo apoptosis, they lose the expression of CD3 ζ as one of the early changes in this process. Therefore, the diminished expression of the CD3 ζ 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

<i>Changes in Signal Transduction</i>	<i>Cancer</i>	<i>Mycobacterial Infections</i>
Decreased CD3 ζ chain expression	+++	+++
Decreased <i>p65^{lck}</i>	+++	+++
Decreased <i>p59^{lyn}</i>	+++	?
Inability to translocate NF- κ B <i>p65</i>	+++	+++
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 Th₁ 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 CD3 ζ chain and *p56^{lck}* tyrosine kinase and were unable to translocate NF- κ B*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 CD3 ζ 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 CD3 ζ , 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 CD3 ζ , 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 CD3 ζ 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 CD3 ζ chain, a decreased expression of *p56^{lck}* tyrosine kinase, and the inability to translocate NF- κ Bp65, 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

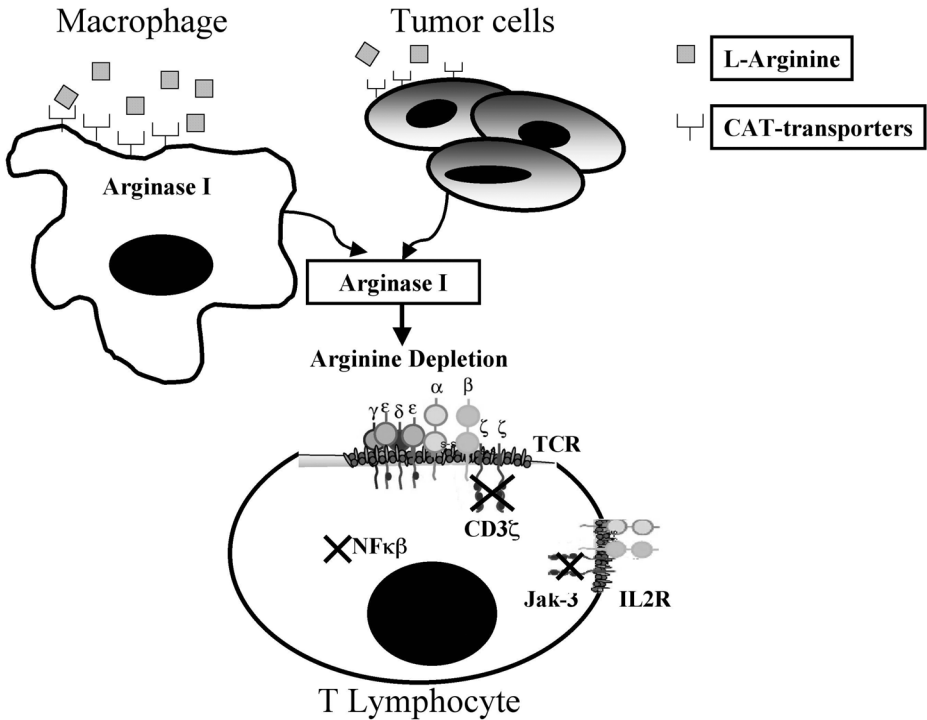


Fig. 1. Arginase produced by macrophages, tumor cells, or microorganisms depletes arginine from the microenvironment and causes alterations in T-cell signal transduction, which results in a state of anergy to the specific antigens.

loss of CD3 ζ 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.

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