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Review

Molecular Immunological Approaches to Biotherapy of Human Cancers – A Review, Hypothesis and Implications*

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Abstract. *The immune system of the human organism comprises the innate system cells and the adaptive immune cells. The former include the hematopoietic cells, mast cells, basophils, monocytes, dendritic cells (DCs) and macrophages, and the latter include $CD4^+$ T cells, $CD8^+$ T cells, T regulatory cells (Tr) and B cells. The innate system DCs are the major antigen-presenting cells to Th_o $CD4^+$ T cells in lymph nodes that polarize into T helper 1 (Th1) and T helper 2 (Th2) cells, which subsequently produce different cytokines. Polarized Th1 cells produce interleukin (IL)-2, IL-12 and interferon (IFN)-gamma, and polarized Th2 cells and the hematopoietic cells produce IL-4, IL-5, IL-6, IL-10 and IL-13. In healthy individuals there is a Th1/Th2 cytokine balance, but during microbial-induced inflammation the pathogens induce an overproduction of the Th2 cytokines that inhibit the adaptive immune response against the pathogen. A review of studies on the Th1/Th2 cytokine balance in humans harboring different tumor types revealed that tumor cells induce increased Th2 cytokine levels in patients' sera that can serve as indicators for the existence of tumors. In this review, studies which correlated the presence of increased Th2 cytokines with the presence of early tumors and tumor progression are discussed. It was suggested that early monitoring of human populations for elevated Th2 cytokines may be used to identify individuals at an early stage of tumor development. A hypothesis is presented which suggests that increased Th2 cytokine synthesis in cancer patients, with early and late tumors, may be treated with Th2 cytokine antagonists. This new approach to cancer treatment will be supplemented by co-treatment with CpG oligodeoxynucleotides(ODNs) which reactivate the adaptive antitumor immune respnse. Studies that provide information on the efficiency of CpG ODN treatment of tumors in mice revealed that tumor regression was achieved by inducing Toll-like receptor 9^+ plasmacytoid dendritic cells (PDCs) to release large amounts of type I interferons (IFN alpha and beta), which inhibit Th2 cytokine synthesis by hematopoietic cells and $CD4^+$ T cells and enhance Th1 cytokine synthesis and activation of the adaptive immunity. It is hypothesized that Th2 cytokine (IL-4 and IL-6) antagonists may be an effective treatment for cancer patients since cytokine antagonists inhibit the increased Th2 cytokines in patients. Such an approach may replace Th2 cytokine monoclonal antibodies, the current treatment for cancer patients. It is hypothesized that the effective treatment of cancer patients with Th2 cytokine antagonists, combined with CpG ODNs, will lead to the inhibition of Th2 cytokines and reactivation of the Th1-induced antitumor adaptive immunity that will destroy tumor cells and cure cancer patients.*

A. Introduction and Aims

a. The relationship between the innate and adaptive immune systems in healthy individuals and cancer patients

Human cancers, as well as plant remedies to treat cancer patients, have been known since 2000 BC. At the beginning of the 20th century, Paul Ehrlich studied the chemical effects of paramidobensol, phenylarsenoxyl, diamido-arsenobenzol and pyocyanase on carcinomas and sarcoma (1). These studies initiated the concept that chemicals can be used to cure microbial infections and human tumors. With the discovery of the structure of DNA by Watson and Crick and the subsequent development of molecular biology during the second part of the 20th century, research on mutations in cellular DNA genomes caused by exposure to

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toxic chemicals and UV or X-rays have led to the understanding of the role of human genes in tumor cell cycle regulation. These studies allowed rational analysis of the sequence of events that leads to tumor development (2). Hanahan and Weinberg (2) indicated that: "tumorigenesis in humans is a multistep process and these steps reflect alterations that drive the progressive transformation of normal human cells into highly malignant derivatives". The authors suggested that: "the vast catalog of cancer cell genotypes is the manifestation of six essential alterations in cell physiology that collectively dictate malignant growth: self-sufficiency in growth signals, insensitivity to growth-inhibitors (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis" (2). The authors concentrated on the intracellular molecular events that transform normal cells into metastatic cancer cells, but did not deal with the impact of tumors on the host cytokine and chemokine networks and the inflammatory processes in the tissue microenvironment of developing tumors.

Parallel to developments in the understanding of the molecular mechanisms governing the gradual alterations in normal cells *in vivo* that lead to malignancy, molecular targets for chemotherapy have been defined, leading to the development of targeted cancer chemotherapy. However, prolonged chemotherapy which caused tumor remission also caused tumor cells to become resistant to anticancer drugs. Tumor remission is replaced by the re-emergence of drug-resistant tumors. Longley and Johnston (3) indicated that: "acquired resistance by tumors is a particular problem, as tumors not only become resistant to the drugs originally used to treat them, but may also become cross-resistant to other drugs with different mechanisms of action. Resistance to chemotherapy is believed to cause treatment failure in over 90% of patients with metastatic tumors." The mechanisms of chemotherapeutic drug influx into tumor cells are not known, but drug-resistant tumors induce expression of the ABC transporter proteins (*e.g.,* P-glycoprotein (PgP) and multidrug resistance protein (MRP) cause drug efflux that cyclosporine inhibits, with toxic effects, while the PgP inhibitors tariquidar and zosuquidar are being tested in phase III clinical trials (3)). Chemotherapeutic drugs and ionizing radiation are the current treatments to stop the development of human tumors. Chemotherapy has led to tumor remission, but the development of drug-resistant relapsing tumors remains an unresolved problem.

During the last twenty years, research on the biology and molecular aspects of the role of the innate and adaptive immune cells of humans has provided a better understanding of the relationships between the early development of a human tumor and the immune system. The innate and adaptive immune cells are the sentinels that collaborate to stop the mutated tumor cells from developing into metastatic cancer cells. Therefore, understanding the mechanisms by which tumor cells evade the host immune systems is the subject of the present review. Current studies on the response of immune system cells to tumors and to their antigens and the response of tumor cells to the host cytokines and chemokines provide the basis for our hypothesis that the gradual development of a tumor into a metastatic entity depends on the tumor's ability to inhibit the development of the adaptive immune response by antitumor cytotoxic T cells (CTLs) and synthesis of antitumor antibodies. Tumors are able to enhance the synthesis of T helper 2 (Th2) cytokines interleukin (IL)-4 and IL-10 by hematopoietic cells, members of the innate immune system. Based on published reports, it is possible that, by treating cancer patients with specific cytokine antagonists and CpG oligodeoxynucleotides (ODNs), inducers of plasmacytoid dendritic cells (PDCs) to release large amounts of type I interferons, the adaptive immune response of the cancer patients will be reactivated to clear drug-resistant and -sensitive tumors and inhibit tumor growth and progression.

b. The human innate and adaptive immune system cells and the Th1/Th2 cytokines

The human immune systems are divided into the innate (ancient) cell system and the adaptive immune system. The innate system cells provide the cytokines which activate the adaptive immune system cells.

1) The innate immune system cells. Figure 1A presents the bone marrow-derived cell types that constitute the human innate immune system:

(a) The monocytes are able to differentiate into macrophages (5), scavengers of dead cell debris and invading microorganisms, and DCs (4), the professional antigen-presenting cells. DCs present in the skin epithelium (Langerhans cells (LCs) (6)) and in all body tissues are the sentinels that capture cancer cell debris and invading pathogens. DCs and LCs use lectin-like DC-SIGN receptors to bind tumor cell debris and microorganisms for engulfment into the cytoplasm and degradation by the proteasomes. The cancer cell antigens are presented by the DC HLA class I and class II molecules to naïve T cells upon arrival of the DCs/LCs in the draining lymph nodes. The PDCs (7) express Toll-like receptor 9 that binds bacterial unmethylated DNA, and release type I interferons (IFN)-alpha and -beta. Monocytes and DCs release Th2 cytokines after activation.

(b) The second type of bone marrow-derived cells, basophils and mast cells (8), are Fc epsilon receptor I-positive ($Fc\varepsilon RI^{+}$) cells that carry cytoplasmic granules containing Th2-type cytokines (IL-4, IL-5, IL-10 and IL-13).

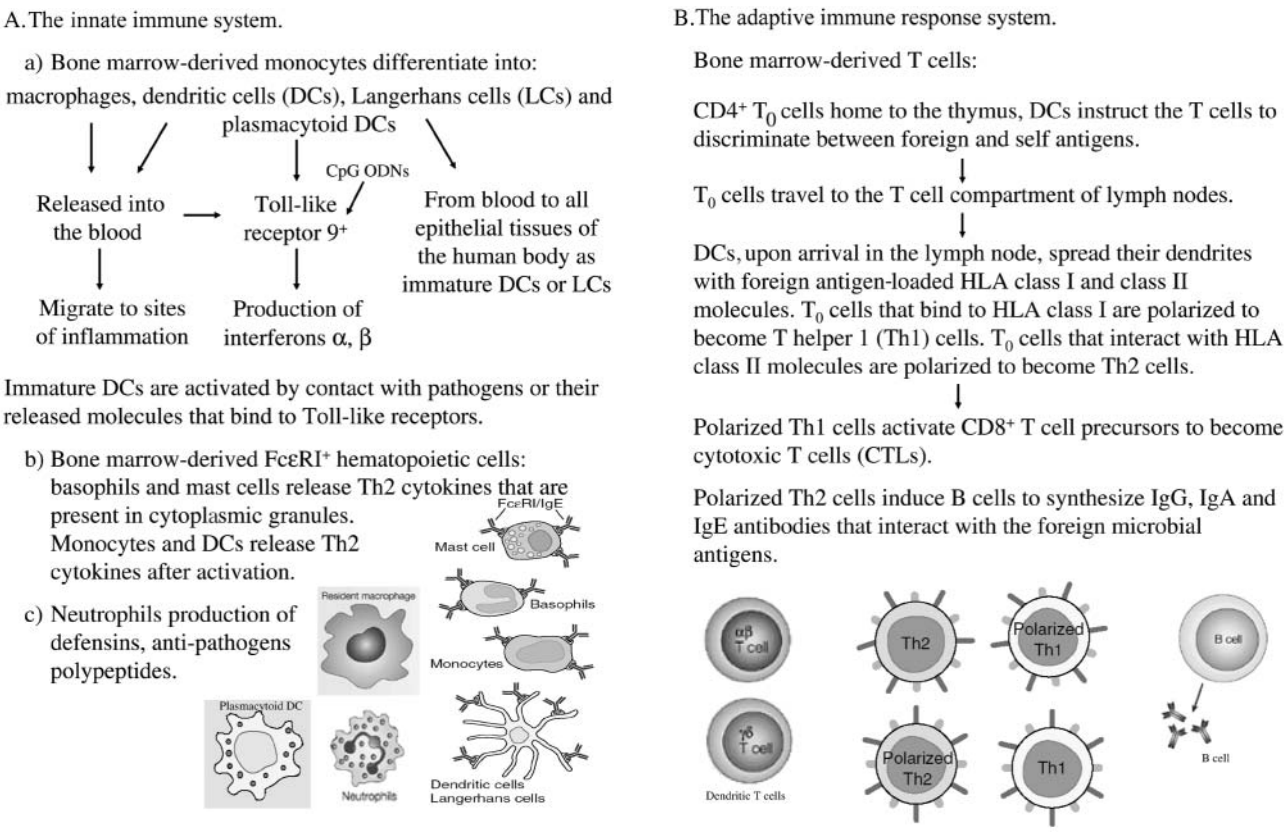

Figure 1. *The innate and the adaptive immune system cells. A. The innate immune system cells; B. The adaptive immune system cells.*

The FcεRI molecules on these cells serve as receptors for the immunoglobulin IgE molecules that are synthesized by B cells of healthy individuals at a very low level.

(c) Neutrophils produce defensins, antipathogen proteins.

2) The adaptive immune system cells. Figure 1B presents the adaptive immune system cell types: a) bone marrow-derived naïve $CD4^+$ T_0 cells travel to the thymus for programming by resident DCs to distinguish self antigens from foreign antigens prior to their migration to the T cell compartments of the lymph nodes, where they reside as naïve Th_0 cells. These cells present T cell alpha/beta receptors (9) on their cell surface which interact with DC HLA class I and II molecules upon arrival in the lymph nodes and are polarized into T helper 1 (Th1) and Th2 cells, respectively. T regulatory cells (Tr) with gamma/delta receptors (9) have a dendritic shape during residence in the skin epithelium. b) The $CD8^+$ T cell precursors are instructed by Th1 cells to become $CD8^+$ T cells, which are the cytotoxic T cells (CTLs) that contain porin which destroys cells which present foreign antigens. c) Natural killer (NK) cells function as scavengers of cells which have lost their HLA class I surface molecules. d) The B cells are another major cell type of the adaptive immune system. These cells are present in the B cell compartments of the lymph nodes and are instructed by Th2 cells to synthesize IgG-specific antibodies to foreign antigens.

3) The transfer of antigenic information by DCs to naïve T (Th0) cells and B cells. The DCs, which are loaded with debris of cancer cells, arrive in the lymph nodes after migration *via* the lymph vessels (in the shape of veiled cells). The DCs spread out their dendrites containing a multitude of HLA class I and II molecules loaded with "foreign" tumor antigens. The Th0 cells that interact with the HLA class I molecules are polarized into Th1 cells, and the Th0 cells that interact with HLA class II molecules polarize into Th2 cells. After polarization, Th1 and Th2 cells differ in their production of cytokines. The Th1 cells are directed to release the cytokines IL-2, IL-12 and interferon gamma, while the Th2 cells release the cytokines IL-4, IL-5, IL-10 and IL-13 (11-16).

The function of Th1 cells is to activate the precursors of the $CD8^+$ T cells to become CTLs capable of clearing the tumor cells. T regulatory (Tr) cells are also induced. In addition to the HLA class I and II genes coding for the HLA proteins that are involved in the presentation of peptides derived from cellular or foreign proteins, CD1

genes are also expressed in humans (CD1A, CD1B, CD1C and CD1D), which enable DCs to present lipid antigens to T cells (17). The polarized Th2 cells induce B cells (10) to synthesize IgG, IgA or IgE antibodies to foreign antigens (Figure 1B), depending on the presence of Th2 cytokines in the blood.

4) The Th1/Th2 cytokine balance in healthy individuals and the role of IL-4 delta 2. Mosmann and Coffman (18) demonstrated that the $CD4^+$ T_0 cells in the lymph nodes are polarized into Th1 cells and Th2 cells after binding to DC HLA class I and class II, respectively. Based on these studies, Kidd (19) presented a hypothesis that the Th1/Th2 differentiation is initiated by antigen-presenting DCs, and a balance between Th1 and Th2 cytokine levels is maintained in healthy individuals. Kidd indicated that: "overactivation of either pattern can cause disease and either pathway can down-regulate the other".

A more detailed analysis of the Th1/Th2 balance in healthy people was presented by Becker (20) (Figure 2). The scheme presents the two T cell systems, Th1 (right) and Th2 (left), that are polarized by interaction with antigen-bearing HLA class I or class II, respectively, on DCs in the lymph nodes. In addition to the polarized Th1 and Th2 cell production of their specific cytokines, two additional cell types are also involved in the regulation of the Th1/Th2 cytokine balance. The PDCs, which are induced by fragmented, non-methylated bacterial DNA that is present in the human intestine to synthesize and release large amounts of type I interferons (alpha and beta). These interferons induce polarized Th1 cells to release IL-2 and IL-12 which activate $CD8^+$ T cell precursors to mature into antigen-specific cytotoxic T cells after interaction with the polarized Th1 cells.

The Th2 cell system includes the $Fc\varepsilon RI^+$ hematopoietic cells, basophils, mast cells, monocytes and DCs which express Th2 cytokines and contain cytoplasmic granules filled with Th2 cytokines (IL-4, IL-5, IL-6, IL-10, IL-13 and IL-21). These cytokines are released into the blood after the binding of allergen-like tumor, microbial-like allergens to the IgE/FcεRI complex. IL-4 and IL-10 inhibit the synthesis of IL-2, IL-12 and IFN gamma by the Th1 cell system. The polarized Th2 cells also produce IL-4 that binds to the IL-4 receptor (IL-4R) alpha molecules that are present on $CD4^+$ T cells and induces them to express the IL-4-responsive genes: IL-4, IL-4 R alpha, HLA class II, FcεRI and I epsilon (precursor for IgE). The binding of IL-4 to its IL-4 R alpha on B cells stops IgG synthesis and enhances the synthesis of large amounts of anti-allergen IgE molecules. These IgE molecules bind to $Fc\varepsilon RI^+$ hematopoietic cells and bind allergen molecules that induce the release of large amounts of Th2 cytokines. However, the increased Th2 cytokine level can be balanced by the release of interferons IFN-alpha and -beta by PDC which

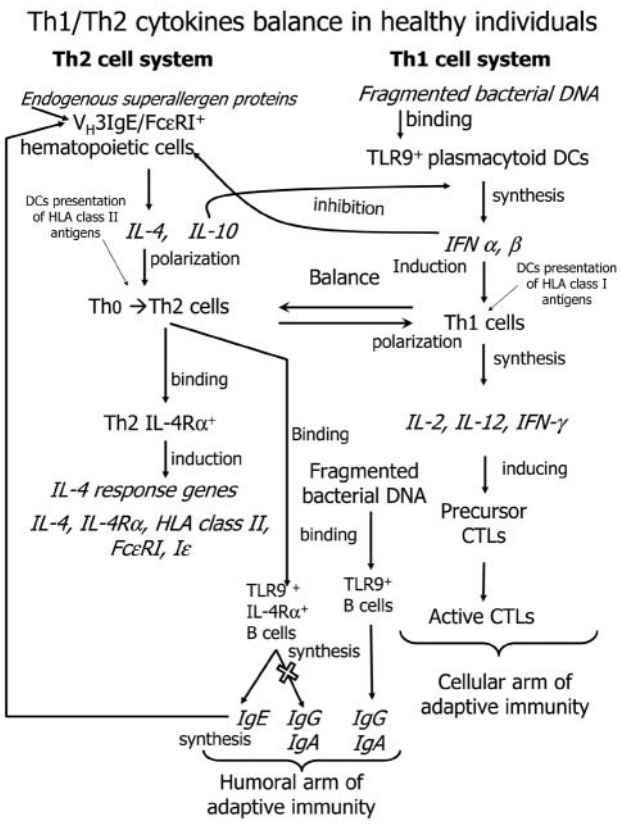

Figure 2. *Th1/Th2 cytokine balance in healthy individuals.*

is induced by bacterial DNA. IFN-alpha and -beta inhibit the release of Th2 cytokines by the allergen-activated hematopoietic cells.

c. Human natural resistance to cancer – a hypothesis

Exposure of humans to various carcinogens culminates in the appearance of a tumor in the human body in some of the exposed individuals, but not all. The reason for this seeming "resistance" to tumors is not known. It is hypothesized that, if indeed the developing and progressing tumors are able to inhibit the patients' adaptive immunity by inducing the gradual increase of high levels of Th2 cytokine, individuals who produce more of the spliced variant of IL-4, lacking the exon 2 of mRNA (designated IL-4 delta 2), than IL-4 may prevent tumor development. IL-4 delta 2 protein conserves the binding domains to the IL-4 R alpha on T cells, but lacks the domains that induce the cells to express the IL-4 response genes, thus serving as a natural antagonist to IL-4. Figure 3 presents two possibilities of the hypothesis (20, 21) for cancer-sensitive and cancer-resistant individuals: (i) When IL-4 is produced at a higher level than

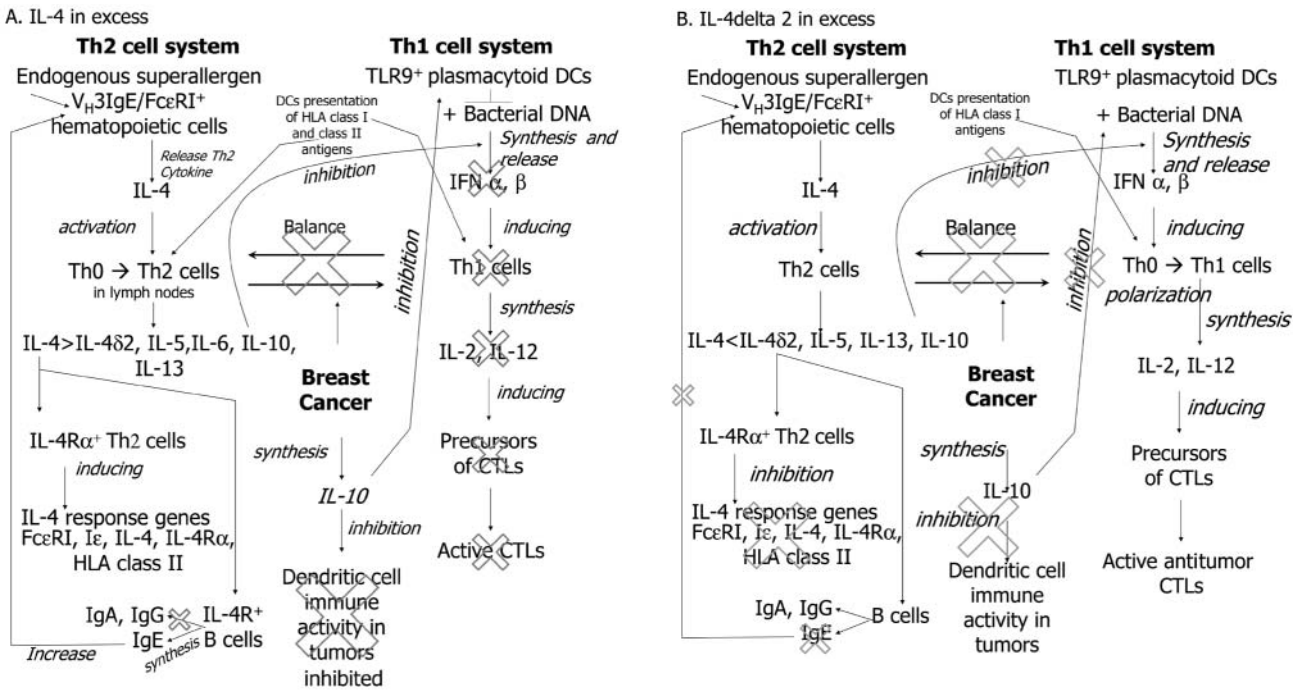

Figure 3. *The impact of the IL-4: IL-4 delta 2 ratio in human serum on the individual's resistance or sensitivity to cancer development. A. IL-4 in excess; B. IL-4 delta 2 in excess.*

IL-4 delta 2 (*e.g.,* 10:1), IL-4 induces the IL-4 response genes in T cells and also shuts off IgG synthesis by B cells and induces IgE synthesis. Under these conditions, the high Th2 cytokine level is dominant in the individual, an allergy-like inflammatory condition develops, and the adaptive immune response is inhibited and tumor development and progession continue. (ii) If the IL-4 antagonist, IL-4 delta 2, is produced in excess of IL-4, the antagonist will block IL-4 activities by occupying the IL-4 R alpha on $CD4^+$ T cells and B cells and the adaptive immune response activity will continue to function. Demissie *et al.* (22) reported that individuals who overexpressed IL-4 delta 2 are able to control the replication of *M. tuberculosis* (TB) in macrophages due to a high level of Th1 cytokines, leading to a latent infection of the bacteria in macrophages. This hypothesis will be discussed together with the hypothesis on a novel approach to anticancer treatment.

d. The aims of the review

The innate and adaptive immune systems function to stop and clear invading microbial pathogens and developing tumor cells to prevent damage to the organism. In these activities, the protective immune systems need to overcome the ability of pathogens and tumor cells to evade the adaptive immune activities by increasing the synthesis of Th2 cytokines inhibitors of the adaptive immune response. The over-production of Th2 cytokines and chemokines induced by the tumor cell allergen-like glycoproteins are used as biomarkers for the existence of a tumor in an individual, providing early detection of the tumor, as suggested by Etzioni *et al.* (23). In this review, attention was given to the involvement of the Th1 cytokines in protection against tumors, and Th2 cytokines as enhancers of tumor progression. Based on the hypothesis that Th2 cytokine antagonists may prevent the inhibition of the adaptive immune response, a new approach to anticancer therapies is discussed.

B. Early Diagnosis and Prognosis of Cancer is Based on Biomarkers for Th2 Cytokine Serum Levels in Humans

Many studies have reported that the levels of IgE, soluble CD23 molecules (sFcεRI) and Th2 cytokines are elevated in cancer patients. These studies revealed that the Th2 cytokines in cancer patients are over-produced.

a. Elevated serum IgE levels in cancer patients

1. Halloten *et al.* (24) compared the hyperimmunoglobulinemia E (IgE) levels in the sera of 143 patients with benign pulmonary disorders to that of 246 healthy adults. Patients with bronchial adenoma had the best prognosis

since the serum IgE levels were not elevated, in contrast to patients with bronchial carcinoma, such as squamous cell carcinoma and small or large cell carcinoma. The authors suggested that: "the increased IgE levels in bronchial carcinoma reflect impaired cellular immunity".

Ownby *et al.* (25) measured serum IgE levels in presurgical sera from 166 non-allergic women admitted to a comprehensive, multidisciplinary study of primary, operable breast cancer. During the follow-up period of 48 months, there were 71 patients with recurrences. The patients were divided into two groups: those with IgE levels greater than the geometric mean of 24 IU and those with levels less than the mean. The rate of tumor recurrences was significantly higher for the IgE more than 24 IU group. IgE was not correlated with other known prognostic factors. The authors concluded that: "the serum IgE level is a significant, independent prognostic indicator in primary breast cancer".

2. Patients with the genetic disorder, hyperimmunoglobulinemia E syndrome (HIES). The genetic disorder of HIES is caused by a Th1/Th2 cytokine imbalance that predisposes the patient to cancer and to pathogen infection. Mosseri (26) reported on a large T-cell lymphoma diagnosed in a 13-year-old girl with HIES. The serum IgE level was >20,000 IU/ml from the age of 6. The authors quote seven additional reports on HIES patients who had developed different tumors: 3 patients with Hodgkin's lymphoma, 2 with large B-cell lymphoma, 1 with histiocytic lymphoma and 1 with Burkitt's lymphoma. One patient with Hodgkin's lymphoma recovered and data were not available for another patient. For 5 patients the outcome was fatal.

Chehimi *et al.* (27) studied 9 patients with HIES who were infected by staphylococci, and 6 controls. It was reported that the HIES patients expressed more IL-2, while chemokines (CXC chemokine, ENA-78, CC chemokines, MCP-3 and eotaxin) were markedly underexpressed. The authors discussed contrasting results showing that IFN-gamma and tumor necrosis factor-alpha (TNF-alpha) production by circulating T cells from HIES patients was defective (28). Borges *et al.* (29) reported a defective IL-12/IFN-gamma pathway in HIES patients.

Shirafuzi *et al.* (30) studied one HIES patient and reported that the ratio of T cells positive for IFN-gamma was significantly reduced compared to controls, supporting the hypothesis that a Th1/Th2 imbalance is involved in HIES.

Netea *et al.* (31) studied a family with 4 HIES patients (38, 37, 30 and 7 years old), who suffered from recurrent skin and respiratory tract bacterial infections, chronic eczema and elevated serum IgE ranging from 5,000 to 16,670 IU/ml. The authors studied the response of blood leukocytes from the HIES patients to heat-killed *S. aureus* or *C. albicans* and noted a severe cytokine imbalance, with a 10- to 30-fold reduction in the IFN-gamma/IL-10 ratio in HIES patients. The authors concluded that: "the imbalance in the Th1/Th2 cytokine production may have been involved in the pathogenesis of the recurrent infection and/or chronic eczema".

It may be concluded that the hyper-production of IgE in HIES patients results from increased levels of the Th2 cytokines IL-4, IL-5, IL-10, IL-13 and the ability of IL-4 to induce B cells to synthesize IgE and not IgG. Increased IgE in HIES patients is an indication of a state of allergy in which the adaptive immune response of the patients is inhibited by the Th2 cytokine IL-4, an inhibitor of Th1 cytokine production by Th1 cells, preventing the activation of the precursor cells to become cytotoxic $CD8^+$ T cells. IL-4 induces B cells to switch to IgE synthesis and inhibits IgG antibody synthesis (Figure 2). Moseri *et al.* (26) reported that an IgE level of >20,000 IU/ml was found in a HIES patient at age 6 and a tumor appeared at age 13. These studies may suggest that prevention of IgE synthesis in HIES patients may prevent the allergy and save the patients' adaptive immune response. This may be achieved by treatment of HIES patients with the IL-4 antagonist IL-4 delta 2.

3. Serum circulatory levels of the soluble CD23 (sCD23) is a prognostic biomarker for B-cell chronic lymphocytic leukemia and non-Hodgkin's lymphoma patients

i) Prognosis of B-cell chronic lymphocytic leukemia (B-CLL). sCD23 (FcεRI or RII) are membrane-bound molecules that are present on B cells, monocytes, IL-4-activated macrophages, eosinophils, platelets and DCs. CD23 is a low-affinity receptor for IgE and is up-regulated by IgE and by IL-4. Molica *et al.* (32) analyzed the prognostic impact of CD23 and sCD23 in an unselected series of 90 previously untreated B-CLL patients and noted that the sCD23 levels allowed two prognostic subgroups with different prognosis to be identified among stage B patients. The authors measured the circulatory levels of sCD23 in the serum of chronic lymphocytic leukemia (CLL) patients and found elevated levels as compared to normal controls. The sCD23 increase reflected the tumor mass as defined by the clinical stage or bone marrow histology. It was noted that life expectancy was significantly shorter in patients with higher circulating levels of sCD23.

Sarfati *et al.* (33) conducted a prospective study to assess the predictive value of the serum sCD23 level on disease progression of 153 CLL patients with a median follow-up of 78 months. It was reported that patients with sCD23 levels above the median value (>574 U/ml) had significantly worse prognoses than patients with lower values (median survival of 53 *versus* 100+ months). Patients with sCD23 above 574 U/ml had a median time to progression of 42 months *versus* 88 months for those with lower levels. The author suggested that patients at stage A of the disease, with a high risk of disease progression based on sCD23, might benefit from earlier therapeutic approaches such as purine nucleoside analogs or bone marrow transplantation for younger patients.

ii) Serum sCD23 level correlates with subsequent AIDS-related non-Hodgkin's lymphoma (NHL). Schroeder *et al.* (34) studied AIDS patients from the multicenter AIDS cohort study (MACS), which included homosexuals and bisexual men who were enrolled at four metropolitan areas in the USA from 1984-5 and 1987-1991. Of the total 5579 MACS participants, 1590 had been diagnosed with AIDS and 167 diagnosed with AIDS-related lymphoma. The authors reported that the serum sCD23 in B-CLL cases increased over time in proportion to the tumor burden. The authors indicated that sCD23 is elevated in patients with Epstein-Barr virus (EBV)-positive NHL in approximately one-half of AIDS patients with NHLs. However, AIDS-positive NHL patients were found to be EBV-positive with lower serum levels of sCD23. In a subsequent study published in the same year, the authors concluded that: "the serum sCD23 does not appear to be mediated by EBV in AIDS NHL patients, but could be related to a pathogenic mechanism of small non-cleaved cell lymphoma" (35).

b. Dysregulation of Th1/Th2 cytokines in human cancer patients and early diagnosis of cancer

The findings reported above reveal that IgE and sCD23 molecules are markedly increased in the serum of certain cancer patients. Since the level of IgE is increased in allergic patients and in HIV-1/AIDS patients, it is suggested that the increased IgE level in cancer patients may be due to induction of the release of IL-4 and synthesis by $Fc\varepsilon RI^+$ hematopoietic cells (basophils, mast cells, DCs and monocytes). This hypothesis was proven to be correct in studies that reported increased Th2 cytokines and decreased Th1 cytokines in patients with different tumors.

1. Th1 and Th2 cytokines in cancer patients. Pellegrini *et al.* (36) studied the levels of IL-2, IFN-gamma, IL-4, IL-6 and TNF-alpha in the sera from a group of colorectal cancer patients and the proliferative response of peripheral bone marrow cells (PBMC) to IL-2 and IL-4 was also studied. The authors reported that the serum levels of IL-4 and IL-6 (Th2 cytokines) were significantly higher than in the normal controls, while the IL-2 level was lower than the control group. These findings led the authors to hypothesize that, in cells of peripheral blood of colorectal cancer patients, there is a dysregulation in the function of Th1 and Th2 cells, probably through IL-4 mechanisms, with an expansion in Th2 cytokines and a malfunction in Th1 cytokines. The author explained the establishment of the initial tumor by an increase in Th2 cytokines which suppressed Th1 cell IL-2 synthesis, resulting in the prevention of activation of $CD8^+$ cytotoxic T cell precursors to develop into antitumor CTLs.

2. Modulation of Th/1Th2 cytokine profiles in advanced head and neck squamous cell carcinoma (HNSCC). Sparano *et al.* (37) measured the concentration of the Th1 cytokine IL-12 and the Th2 cytokines IL-4, IL-6 and IL-10 in the plasma of 58 patients with histologically proven HNSCC. The plasma cytokines were compared in patients by T_1/T_2-stage *vs.* T_3/T_4-stage tumor and in patients with or without nodal metastatic involvements. It was reported that the concentrations of IL-12 were greater in the sera from patients without nodal metastases and with T_1/T_2-stage tumor. The IL-10 levels were greater in patients with nodal metastases and with T_3/T_4-stage tumors. The IL-6 concentrations were greater in T_3/T_4-stage tumors. It was concluded that advanced HNSCC patients (T_3/T_4 stage and metastases) have diminished Th1 immune response and a stronger Th2 cytokine response when compared with patients at the T_1/T_2 stage.

3. Th1/Th2 cytokines in patients with pulmonary carcinoma. Yamazaki *et al.* (38) obtained serum samples from 68 Japanese patients with pulmonary adenocarcinoma before the operation and measured the serum levels of IFN-gamma and IL-4. Eighteen out of 22 patients were classified as predominantly IFN-gamma-positive. This group was free from lymph node metastases. Of the Th2 cytokine-positive group, 5 out of 7 patients had lymph node involvement. Sixty-two patients underwent complete tumor resection and 9 out of 19 patients in the Th1 group developed recurrences, while no relapse of the tumor appeared in the Th2 group during the observation period. The disease-free interval for the Th2 group was significantly longer than that for the Th1 group (44.2 months *vs.* 26.1 months, $p=0.03$). The authors noted that the profiles of Th cytokines showed a significant relationship with age and gender. Patients 70 years old or older had significantly lower serum levels of IL-4 and 14 out of 36 patients older than 70 years produced both IFN-gamma and IL-4. The IL-4 serum level was higher in women than in men. The female-to-male ratio, higher in the Th2 group and lower in the Th1 group, is based on steroids since estrogen enhances humoral immunity and depresses the cellular response.

Ito *et al.* (39) evaluated the ratios of Th1 to Th2 and T cytotoxic cells Tc1 to Tc2 among peripheral blood lymphocytes (PBL), regional lymph node lymphocytes (RLNL) and tumor infiltrating lymphocytes (TILs) in 46 non-small cell lung carcinoma patients with and without recurrences after surgery, and in 29 patients with lung carcinoma. PBL from normal volunteers served as controls. The authors reported that the Th1/Th2 and Tc1/Tc2 ratios were significantly elevated in tumor tissues, but were depressed in patients with tumor recurrences. It was concluded that a favorable Th1- and Tc1-dominant pathway is induced in the tumor tissue of operable patients, but a shift toward Th2/Tc2 takes place with progression of the disease.

4. Tumor-specific infiltrating T cells (TILs) in ovarian and breast cancer produce Th1 and Th2 cytokines. Goedegebuure *et al.* (40) generated CTL cultures from tumors of ovarian cancer patients and from 3 breast cancer patients. All CTL lines were T cell receptor (TCR) alpha/beta-positive and predominantly $CD8^+$. The CTLs released IFN-gamma and TNF-alpha, but not IL-4. The authors concluded that: "the secretion of Th1-like cytokines, as opposed to Th2 cytokines, suggests a potential for the enhancement of the endogenous (adaptive) immune response to the tumor".

Kozlowski *et al.* (41) reported that the concentrations of IL-6, IL-8 and IL-10 in blood serum of breast cancer patients were higher than in healthy women and correlated with the clinical stage of the breast cancer. The authors concluded that the elevated IL-6, IL-8 and IL-10 concentrations in the sera of breast cancer patients could be used to identify patients with a poor prognosis who may benefit from more aggressive chemotherapeutic anticancer treatment.

5. Th1 and Th2 cytokines in patients with prostate cancer. Filella *et al.* (42) studied the expression of IL-2, IFN-gamma, IL-4 and IL-10 in $CD8^+$ and $CD4^+$ lymphocytes, by flow cytometry, in 12 patients with prostate cancer and in 7 healthy subjects. It was reported that, in relation to the healthy subjects, an increase in IL-10 expression and a decrease in IL-2 expression was observed, leading to the conclusion that the Th2/Th1 disequilibrium may be implicated in the evolution of the tumor.

6. Th1/Th2 cytokine imbalance in patients with lymphoma, leukemia and thymoma. Mori *et al.* (43) evaluated, by intracellular cytokine analysis, the Th1/Th2 cytokine balance in peripheral blood Th cells in 19 patients with previously untreated B-cell diffuse large cell lymphoma (DLCL) and 18 patients with DLCL with complete remission (CR). The authors reported that the mean percentages of Th2 cells among the $CD4^+$ cells in DLCL patients, and Th1 cells in $CD4^+$ cells from CR patients, were significantly increased compared to healthy volunteers. The mean Th1/Th2 ratio was significantly lower in DLCL compared to CR patients. The authors concluded that: "the Th1/Th2 balance was polarized to Th2 in untreated DLCL patients and to Th1 in CR patients, which suggests that Th1/Th2 imbalance could play a role in lymphomagenesis and durable remission, respectively".

Podhorecka *et al.* (44) studied 30 patients with newly diagnosed untreated B-CLL and 12 healthy individuals. The patients were classified into early stages 0-I and advanced stages II-IV. PBLs were isolated and the three-color flow cytometry technique was used to identify IL-4 and IFN-gamma-expressing cells. It was reported that the dominance of Th1 cells and CTL-mediated immunity in B-CLL patients was shifted towards Th2 cytokines, revealing Th2 cell dominance during disease progression.

Fujisao *et al.* (45) studied T helper sets in the PBLs of a 66-year-old woman with acquired pure red cell aplasia and thymoma by flow cell cytometry determination of IFN-gamma and IL-4 in the cytoplasm of the peripheral $CD4^+$ T cells. The percentage of Th1 and Th2 was counted by FACS and the Th1/Th2 balance was evaluated by the ratio of % IFN-$gamma^+$ to % $IL\text{-}4^+$ cells. Before thymectomy, the Th1/Th2 ratio was as low as 3.4, but after the thymectomy it rose to 6.8 and was accompanied by hematological improvement. When a decrease in hematopoiesis returned, the Th1/Th2 ratio declined to 4.9. Treatment with cyclosporine A therapy dramatically improved hematopoiesis, with a concomitant steep rise of the Th1/Th2 ratio to 8.6.

7. Determination of IL-10 content in the sera of cancer patients as an independent prognostic marker for cancer.

i) IL-10 in serum of metastatic melanoma patients. Dummer *et al.* (46) studied the IL-10 serum levels in 104 untreated patients at different stages of melanoma, who were compared to healthy subjects, and 22 patients with inflammatory dermatoses as controls. Only 1 out of 31 patients with stage I melanoma (3%) and 1 out of 2 stage II (6%) showed detectable IL-10 levels. However, 6 out of 17 patients with lymph node metastases (stage III, 35%) and 29 out of 40 patients with widespread disease (stage IV, 73%) had IL-10 levels ranging from 15 to 480 pg/ml, contrary to healthy persons. One control patient had a detectable IL-10 serum level. It was concluded that IL-10 in melanoma patients contributes to the inhibition of the antitumor immune response *in vivo*.

Nemunaitis *et al.* (47) studied the serum IL-10 level of 41 melanoma patients and 50 normal volunteers and reported that the median IL-10 level in melanoma patients was 8.75 pg/ml compared to <3.0 pg/ml in normal volunteers. It was also found that the survival of melanoma patients with IL-10 levels of above 10.0 pg/ml was 365 days, compared to 557 days for melanoma patients with IL-10 levels lower than 10.0 pg/ml. Thus, elevated IL-10 levels correlated with poor survival.

Lauerova *et al.* (48) studied Th1 and Th2 cytokines and soluble IL-2 receptor (sIL-2R) levels in 26 malignant melanoma patients in phase III prior to and during adjuvant immunotherapy. The control group consisted of 26 healthy persons. The patients were treated with recombinant IFN-alpha according to the EORTC melanoma group protocol and the cytokines were quantified in patients' sera using commercial ELISA kits. It was reported that the melanoma patients had significantly lower levels of IL-2 and IFN-gamma and pathologically elevated levels of the cytokines IL-4, IL-6 and IL-10 compared to healthy subjects,

suggesting a Th1/Th2 imbalance. The authors noted increased IL-2 and IL-15 cytokine levels in some patients, while patients who manifested early relapse during immunotherapy had a marked decrease in IL-2 and IL-12 levels compared to patients without remission, showing elevation of TNF-alpha and sIL-2R over 10 months of immunotherapy. The authors suggested that measurements of Th1/Th2 cytokines may be useful as early signals of disease deterioration requiring evaluation of the need for immunotherapy.

ii) Serum IL-10 levels in the prognosis of advanced non-small cell lung cancer (NSCLC) patients. De Vita *et al.* (49) measured serum IL-10 levels in blood from patients before chemotherapy, on completion of therapy and at follow-up, and analyzed the results in comparison with other prognostic variables, developing a model predicting survival and time to treatment failure. Sixty stage III or IV patients undergoing conventional platinum-based regimens were studied. The authors reported that the serum IL-10 levels were elevated in cancer patients compared to healthy people. Patients with metastatic disease showed significantly higher levels of IL-10 than patients with undisseminated cancer. Following the completion of treatment, the patients were classified as responders if they had achieved complete response, partial response, or stable disease. Non-responders were patients with progressive disease and a significant IL-10 increase in the serum, while the IL-10 serum values significantly decreased in responders. The authors concluded that IL-10 measurement has independent prognostic utility and may be useful for the detection of disease progression.

Hatanaka *et al.* (50) examined IL-10 and IL-10 receptor (IL-10R) mRNA in 82 NSCLC by RT-PCR assay and reported that 68 out of 82 (83%) NSCLC surgical specimens (40/50 adenocarcinoma, 22/26 squamous cell carcinoma, 5/5 large cell carcinoma and 1/1 adenosquamous cell carcinoma) revealed intracellular IL-10 mRNA. RT-PCR also revealed IL-10R mRNA in 79 out of 82 cases of NSCLC (96.1%). In 12 patients serum IL-10 was not elevated. Patients with IL-10 production showed significantly poorer prognosis than those without IL-10 production. The authors concluded that cytoplasmic IL-10 correlated with the clinical prognosis.

Neuner *et al.* (51) reported that 14 NSCLC patients with surgical resection of the tumor and high IL-2 at diagnosis had a median survival of 86.2 months, while 19 patients after surgical resection had low IL-2 at diagnosis and their median survival time was 11.3 months. Thus, IL-10 inhibition of IL-2 has prognostic significance for survival.

iii) IL-10 is an independent predictor in patients with metastatic renal cell carcinoma (MRCC). Wittke *et al.* (52) evaluated the pretreatment serum level of IL-10 and its prognostic value for 80 metastatic renal cell carcinoma (MRCC) patients. The authors reported that an elevated serum level of IL-10 in pretreatment MRCC patients was a predictor for MRCC, suggesting a potential role of IL-10 in the development of advanced renal cell carcinoma and the need for therapeutic treatments.

Negrier *et al.* (53) also reported that increased levels of IL-6 in the sera of MRCC patients appeared to be an independent prognostic factor.

iv) Serum levels of IL-10 and sIL-2R as prognostic markers for the transition from adenoma to colorectal cancer. Berghella *et al.* (54) studied a group of healthy subjects, subjects with adenoma (the precursor of colorectal cancer) and colorectal cancer patients to identify peripheral blood invasiveness markers during the progression from normal mucosa through adenoma to colorectal tumor. The authors evaluated the relationships between serum levels of IL-2, sIL-2R, IFN-gamma, IL-4, IL-6, IL-10 and sICAM-1. The results indicated that in normal mucosa through the adenoma stage to tumor progression, the host immune response proceeded from an initial Th1-mediated immune response to a Th2 immune-suppressive response during the stages of adenoma and colorectal tumor. During the adenoma stage, IL-10 and sIL-2R were not involved, but these bio-marker levels increased in the cancer patients. Thus, IL-10 and sIL-2R are prognostic during the progression of an adenoma to become a metastatic tumor.

v) Concentrations of IL-4 in sera of patients with early metastatic solid neoplasms. Lissoni *et al.* (55) evaluated the levels of serum IL-4, IL-6 and IL-10 in a group of 50 cancer patients, 28 of whom showed distant organ metastases of lung and gastrointestinal cancers. A control group consisted of 60 healthy subjects. It was reported that the patients did not have high levels of IL-4 and concluded that the high levels of IL-6 and IL-10 were produced by macrophages and not T cells in the tumor microenvironment.

vi) IL-10 and IL-4 production by basal (BCC) and squamous cell carcinoma (SCC). Kim *et al.* (56) explored the possibility that tumors may alter the host immune response by releasing immunomodulatory cytokines, and studied skin tumors, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) by RT-PCR. The authors detected cytokine mRNAs synthesized by the SCC tumor cells and by TIL that were derived from biopsies from the patients suffering from either one of the two tumors. It was reported that IL-10 was expressed in SCC tumors and not in benign tumors, and was present in the cultured tumor cell supernatants. IL-10 mRNA was strongly expressed in BCC cell lines, while the TILs expressed IL-4 and IL-2 and IFN-gamma mRNAs. Treatment of BCC tumors by intralesional IFN-alpha induced tumor regression with concomitant up-regulation of IL-2 and down-regulation of IL-10 mRNA expression in the tumor. The authors concluded that: "tumor production of IL-10 may provide a mechanism of evading the local cell-mediated immune response".

8. Early detection of epithelial ovarian cancer (EOC). Mor *et al.* (57) used blood from 86 women: 28 healthy women, 18 women newly diagnosed with epithelial ovarian cancer (EOC) and 40 patients with recurrent disease (stage III/IV). The average age of the disease-free individuals was 60.8 years and of the EOC patients 57.1 years. The authors reported a blood test based on the simultaneous quantification of 4 analytes (leptin, prolactin, osteopontin (OPN) and insulin-like growth factor II). The authors used microarray analysis to determine the levels of 169 proteins in the serum of the subjects and found 4 proteins (leptin, prolactin, osteopontin (OPN) and insulin-like growth factor-II (IGF-II)) that could discriminate between disease-free and cancer patients, including patients diagnosed with stage I/II EOC. To evaluate the significance of their findings, the authors evaluated, in a blind manner, the levels of the 4 proteins in 106 healthy women and 100 patients (24 stage I/II and 76 stage III/IV). The authors concluded that: "no single protein could completely distinguish the cancer group from healthy controls". The final results of the test showed a sensitivity of 95%, a specificity of 95%, a positive predictive value 95% and a negative predictive value of 94%, in the diagnosis of EOC.

9. Evaluation of the studies on the Th1/Th2 imbalance in cancer patients. The above studies, summarized in Table I, provide a preliminary analysis of the skewed Th1/Th2 cytokine balance toward the Th2 cytokine and the production of large amounts of Th2 cytokines as their mechanism to inhibit the antitumor adaptive immune response. The increased level of serum Th2 cytokines and their cellular receptors in cancer patients were suggested to be early biomarkers of the existence of foci where a tumor is developing. Early detection of tumors at the adenoma stage can detect early tumor development long before the appearance of metastases and tissue damage. The use of biomarkers to identify different human tumors may open a new avenue for the development of effective anticancer treatments, as suggested below.

c. Implications of the studies on the prognostic values of the cancer biomarkers for tumor detection and treatment

The above studies reporting on the increased levels of IgE, sCD23 and Th1/Th2 cytokines (tumor biomarkers) that are present in the serum of patients harboring different tumors are presented in Table I. The information is not complete since the Th1/Th2 cytokine levels in many additional cancers have not been reported. On the basis of the reported biomarkers, the human tumors studied may be divided into 3 subgroups (A-C). Subgroup A includes 6 tumors: breast cancer, advanced HNSCC, BCC, SCC, large T-cell lymphoma, DLCL and prostate cancer. During tumor progression, these tumors caused a marked increase of Th2 cytokine serum levels that inhibit the host anticancer adaptive immune response. In breast carcinoma and large

Table I. *Prognostic value of cancer biomarkers in the blood of cancer patients.*

Human tumors	Elevated level of biomarkers in cancer patients sera			
	IgE increase	Increase sCD23 (IgERI)	Dysregulation Th1<Th2 cytokines	IL-10, IL-6 increase
A				
Breast cancer	+ (25)*		+ (40)	IL-6 + (60, 61) IL-10 + (41)
Advanced head & neck squamous cell carcinoma			+ (37)	
Basal cell carcinoma			+ (56)	
Squamous cell carcinoma			+ (56)	
Large T-cell lymphoma	+ (26)			
B-cell diffuse large cell lymphoma			+ (43)	
Prostate cancer			+ (42)	
B				
Non-Hodgkin's lymphoma		+ (34)		
Small non-cleaved cell lymphoma		+ (35)		
B-cell chronic lymphocytic leukemia		+ (32, 33)		
C				
Metastatic melanoma			+ (48)	IL-10 + (46, 47)
Metastatic renal cell carcinoma				IL-10 + (51, 52, 53)
Colorectal cancer			+ (36)	IL-10 + (53, 54)
Advanced non-small cell lung carcinoma			+ (51)	IL-10 + (49)
Ovarian cancer			+ (40)	IL-10 + (57)

*References

T-cell lymphoma, increased levels of IgE were also detected, indicating that the increased IL-4 cytokine level induced B cells to stop IgG synthesis and switch to IgE synthesis, causing inhibition of the host adaptive immune response that allows the tumor cells to proliferate and progress to become metastatic.

Subgroup B includes 3 tumors: non-Hodgkin's lymphoma, small non-cleaved cell lymphoma and B-CLL, which induce increased levels of sCD23 in the patients' sera. The sCD23 protein is the soluble IgE receptor FcεRI, suggesting that this biomarker is released from the hematopoietic cells which parallel the increase in IgE in the serum, similar to tumors in subgroup A.

Subgroup C includes 5 tumors: metastatic melanoma, MRCC, colorectal cancer, advanced NSCLC and ovarian carcinoma. These tumors resemble subgroup A in their ability to cause Th1/Th2 cytokine imbalance in the patients' sera. In addition, these tumors produce large amounts of the Th2 cytokine IL-10 into the tumor microenvironment, after stimulation by the cytokine IL-6 produced by macrophages that are present in the tumor microenvironment.

The preliminary analysis (Table I) provides the basis for the development of early diagnostic tests to identify, in the human serum, the possible existence of tumors at an early stage of development. The presence of increased levels of the Th1 (IL-2, IL-12, IFN-γ) and Th2 cytokines (IL-4, IL-6, IL-10), IgE and sCD23 in a patient's serum as biomarkers for cancer means that the possibilities that the tested individual is not allergic or infected by HIV-1 (58) allergy-inducing viruses need to be ruled out. The use of antibody microarray for Th2 cytokine detection and additional analyses may provide the basis for early anticancer treatments of tumor-bearing individuals. The tumors listed in subgroup C produce and release IL-10 into the tumor microenvironment. IL-10 synthesis is regulated by macrophages and IL-6 present in the tumor microenvironment. The reported results (Table I) reveal two mechanisms by which different tumors inhibit the Th1 cytokine synthesis and the cellular arm of the adaptive immune response: i) systemic skewing of the Th1/Th2 balance toward Th2 cytokines involving the hematopoietic cells, and ii) IL-6 induction of tumors to release IL-10 locally in the microenvironment.

C. Tumor Cells Respond to Chemokines and Produce Th2 Cytokines to Evade the Host Adaptive Immunity in the Tumor Microenvironment

Tumors do not rely only on the systemic Th2 cytokine increase and the inhibition of the adaptive anticancer immunity, since innate and adaptive immune cells infiltrate into the tumor microenvironment. Many tumors produce IL-10, the inhibitor of Th1 cytokine synthesis. This property of tumors ensures the local evasion of the adaptive immunity and the DCs, $CD4^+$ and $CD8^+$ T cells in the microenvironments. The following reports provide the mechanism of the local evasion of the adaptive immune response.

1. Human tumor cells produce cytokines to evade the host adaptive immune response

a. Breast tumors

(i) Dendritic cells are dysfunctional in patients with breast cancer. Satthaporn *et al.* (59) studied the DCs that were isolated from PBLs from breast cancer patients and LNDCs after surgery. It was reported that 70-75% of purified DCs that were isolated from patients with operable tumors demonstrated reduced IL-12 p40 production by T cells when incubated with purified DCs. Decreased expression of HLA-DR, CD-40 and CD-86 (DC activation markers) were noted on DCs from tumor-bearing women. The reason for this phenomenon was not reported.

(ii) Local feedback mechanisms by hormones and cytokines in breast cancer. Singer *et al.* (60) suggested that, since the development and function of the breast are controlled by local (hormones) and systemic (cytokines) signals, these signals may also control the development of breast cancer. One of the studies by Crichton *et al.* (61) reported that estrogens markedly enhanced IL-11 expression in adipose stromal cells in breast tissues. IL-11 and other class I cytokines are potent stimulators of aromatase-P450 expression in human breast fibroblasts. Estradiol, made by aromatase in fibroblasts, leads to stimulation of adjacent tumor cells and results in up-regulation of IL-11.

Jablonska and Pietrustka (62) reported that unstimulated and stimulated polymorphnuclear cells (PMNs) and whole blood cells (WBC) from breast cancer patients showed decreased ability to release soluble IL-6 receptor (sIL-6R) in comparison to a control group. The mean concentrations of sIL-6R and IL-6 in the sera of breast cancer patients were higher than those in the control group.

Blais *et al.* (63) studied the potential action of IL-4 and IL-13 on breast cancer cells and reported that exposure of the ZR-75-1 breast cancer cell line to IL-4 and IL-13 for 10 days decreased the mitogenic action of 17-estradiol by 75% and 55%, respectively, with no change of basal cell proliferation.

b. Malignant melanoma

(i) Recruitment of immature plasmacytoid dendritic cells (PDCs) and myeloid DCs to the tumor microenvironment. Vermi *et al.* (64) analyzed the phenotype and distribution of DCs in primary cutaneous melanoma and lymph nodes by immunohistochemistry. It was reported that, in primary melanoma tumors in the skin epidermis, an increased number of DCs ($CD1a^+$/Langerhans $cell^+$) was noted. Peritumoral DCs included a large population of DC-$SIGN^+$/mannose $receptor^+$/$CD1a^-$ DCs, a small subset of

$CD1a^+$ DCs and plasmacytoid monocytes/plasmacytoid DCs (PM/PDCs). The authors suggested that the PM/PDCs, the major producers of type I interferons, were recruited by SDF-1 secreted by the tumor cells. However, the expression of the IFN-alpha-inducible protein MxA was very limited and all DC subsets were predominantly immature. The peritumoral T lymphocytes were with poor T cell stimulation. The authors concluded that the peritumoral and infiltrating DCs indicate defective maturation due to the primary cutaneous melanoma.

(ii) Metastatic melanoma escapes the immune surveillance by expressing and releasing IL-10. Enk *et al.* (65) studied the function of melanoma tumor-derived DCs in patients with treatment-responding (rM) or progressing (pM) melanoma metastases. $CD83^+$ DCs were purifed freshly from large rM or pM tumor metastases following chemoimmunotherapy. It was reported that the DCs were five times more potent inducers of allogeneic T cell proliferation than control DCs. Analysis of the culture supernatants from PM tumors revealed the synthesis and release of the Th2 cytokine IL-10, whereas rM tumors released the Th1 cytokines IL-2, IL-12 and IFN-gamma. The authors concluded that: "melanoma-derived factors convert DC-antigen presenting function to the induction of tolerance against tumor tissue, changing tumor DCs to "silencers" of anti-tumoral immune response".

Steinbrink *et al.* (66) reported that immature DCs exposed to IL-10 for 2 days showed a strongly reduced capacity to stimulate a $CD4^+$ T cell response, while mature DCs were resistant to the effect of IL-10. Ludewig *et al.* (67) reported that apoptosis of DCs was strongly enhanced by IL-10.

(iii) Role of IL-8 in metastatic human melanoma. Bar-Eli (68) reviewed the studies on the production of IL-8, a member of the superfamily of CXC chemokines, by melanoma tumors and its correlation with metastasis. The author concluded that IL-8 also serves as a very potent angiogenic factor for melanoma cells, possibly through the activation of the collagenase type IV metalloproteinase MMP-2, and that IL-8 may serve as an angiogenic factor, distinguishing benign from malignant cells.

c. Renal cell carcinoma (RCC) cell lines. Meuetrier-Caux *et al.* (69) used renal cell carcinoma (RCC) cells *in vitro* to test their effect on antigen presentation by DCs. The RCC cell lines were found to release IL-6, that inhibited the differentiation of $CD34^+$ cells into DCs. The authors concluded that: "the inhibition of DC development could represent a mechanism by which tumor cells will escape immune recognition".

d. Pancreatic carcinoma. Ebrahimi *et al.* (70) studied the serum levels of vascular endothelial growth factor (VEGF) and IL-1 alpha and beta in sera from 51 patients with pancreatic carcinoma and from 48 to 62 healthy volunteers and found that the above-mentioned cytokines were not elevated in the cancer patients. In contrast, the cytokines IL-6, IL-8, IL-10 and IL-1R antagonist (IL-1RA) were significantly elevated as compared to the controls. Patients who had IL-6 levels >5.2 pg/ml or IL-10 levels >9.8 pg/ml had significantly worse survival compared to patients who had lower IL-6 and IL-10 levels. Patients with IL-1RA levels of <159 pg/ml had significantly worse survival rates compared to patients with higher IL-1RA levels. High IL-6, IL-10 and IL-8 were associated with poor performance status and/or weight loss. The authors concluded that the determination of serum cytokine levels may provide a useful prognosis.

e. Multiple myeloma. Lauta (71) reviewed studies on the cytokine network in multiple myeloma. Production of IL-6 by bone marrow stromal cells is controlled by IL-1 beta and IL-10. IL-6 induced differentiation of myeloma plasmablastic cells into mature plasma cells that was influenced by IL-3 and IL-4. Increased levels of IL-6 and sIL-6R, together with IL-3 and IL-4, may be used as diagnostic markers to correlate with disease activity and tumor stage. The author indicated that interferons are mainly used in immunotherapy for multiple myeloma, but additional clinical trials are required to evaluate the effectiveness of anti-IL-6 antibody therapy.

1. Macrophage-derived IL-6 induces IL-10 production by colon carcinoma cells. Herbeuval *et al.* (72) investigated the interactions between macrophages and tumor cells in humans by *in vitro* culturing of macrophages from pleural effusions of patients with malignancies and from healthy people. The macrophages from both sources strongly stimulated IL-10 production by three different human colon adenocarcinoma cell lines. Recombinant IL-6 (rIL-6) also stimulated the secretion of IL-10 by the colon tumor cells, while monoclonal antibodies (mAb) against IL-6 and IL-6 receptor (IL-6R) prevented this effect. The IL-10 gene regulation was mediated by STAT3, which was phosphorylated after IL-6 binding to its receptor, IL-6R.

It is of interest that macrophages in the tumor microenvironment are the source of IL-6 that induces the tumor cells to synthesize and release IL-10, inhibitors of the infiltrating innate system cells, monocyte-derived antigen-presenting DCs and PDCs, the major producers of interferon type I (IFN-alpha and IFN-beta). With the help of IL-10 the tumor cells are protected from the PDCs.

2. Chemokines and their receptors influence the development of tumors and their metastases. Balkwill (73) reviewed the relationship between the host chemokine network and the ability of a tumor to develop metastases. The chemokines contribute to the polarization of Th2 cells and the cytokine receptor CXCR4 is expressed on 23 different cancer types, together with additional chemokine receptors which are

associated with increased metastatic capacity. Preliminary laboratory data from this study showed that chemokine receptor antagonists inhibit macrophage infiltration into the tumor microenvironment and can induce apoptosis of the tumor cells and prevent metastatic spread.

D. Natural and Synthetic Inhibitors of Th2 Cytokine Can Reactivate the Host Adaptive Immunity to Clear the Tumor

a. Anti-cytokine antibody therapies

Human cancers are classified according to the tissue or organ in which they develop (2) as a result of mutations caused by intrinsic or extrinsic agents. Usually, the human tumor is detected at a relatively late stage of its development, when the cell mass of the tumor is discernible. The cancer patient's Th1/Th2 cytokine balance is already skewed toward Th2 cytokines and the adaptive immune response is partly or completely inhibited. In many tumors, metastatic tumor cells invade essential organs and cause the patient's demise. Many studies reported that testing the Th1 and Th2 cytokine levels in the serum of seemingly healthy individuals may identify individuals with increased Th2 cytokine levels in the serum who will need further diagnostic tests for the identification of a tumor at an early stage.

Based on the demonstration that tumor cells produce and induce increased serum levels of Th2 cytokines, drugs that reduce the level of the Th2 cytokines in the serum of cancer patients were developed. The use of human monoclonal antibodies against the Th2 cytokines (74, 75) is the first attempt to prevent the damaging effects of Th2 cytokine on the cancer patient's adaptive immune response.

1. Treatment with anti-TNF antibodies as anticancer therapy. Mocellin *et al.* (76) reviewed the molecular and biological activities of TNF as an antineoplastic and tumor-promoting factor and anti-TNF therapy for cancer. The authors noted that a large body of evidence supports the antineoplastic activity of TNF, showing TNF-mediated tumor regression, while some preclinical findings suggested that TNF may promote cancer development and progression. The authors reviewed the experimental and clinical evidence for anti-TNF therapies. Two types of molecules are licensed for clinical use: a) Etanercept (two molecules of the extracellular portion of the human TNF-R-Fc (fused to the Fc portion of human IgG), and b) Infliximab and adalimumab (fully humanized monoclonal antibodies specific to TNF). Tsimberidou *et al.* (77) reported on a pilot study with recombinant TNF-R-Fc (Enbrel) in patients with refractory multiple myeloma. Studies on TNF antagonists carried out on patients with advanced melanoma or RCC with high-dose intravenous *i.v.* IL-2, with or without a TNF antagonist, revealed that there was no effect on the tumor response rate or the severity of side-effects. A review of the preliminary reports on breast cancer patients treated with Etanercept alone revealed no evidence of clinical response.

b. Natural splice variants of cytokines and their potential for treatment of cancer patients – a hypothesis

The above attempts to use humanized monoclonal antibodies to Th2 cytokines to decrease their damaging impact on the cancer patient's adaptive immune system is based on the findings that the Th1/Th2 balance in the cancer patient is skewed toward the Th2 cytokines to which the tumor cells contribute IL-10 that inactivates the innate system DCs. Therefore, reducing the Th2 cytokine levels with antibodies may have a temporary effect, since the tumor cells continue to proliferate and to produce more Th2 cytokines. It was reported that during the transcription of the Th2 cytokines IL-4 and IL-6 genes, two or more mRNA species are transcribed from the two genes which code for splice variants IL-4 delta 2, IL-6 delta 3 and IL-6 delta 5, respectively, which function as antagonists to the full-length IL-4 and IL-6 due to their ability to bind to the respective receptors on different cell types. It is suggested that these splice variants may be developed as IL-4 and IL-6 antagonists for the treatment of cancer patients. Injection of the IL-4 and IL-6 antagonists at concentrations higher than their levels in the serum of cancer patients will prevent damage to the anticancer adaptive immunity by binding to their natural receptors.

A second approach to cancer therapy, which is currently being explored, is the use of CpG ODNs which mimic unmethylated bacterial DNA binding to TLR9 receptors on PDCs that induce the release of large amounts of type I IFN-alpha and -beta, inhibitors of the synthesis of Th2 cytokines and inducers of Th1 cytokine synthesis and the activation of CTL precursors into antitumor CTLs.

A third approach is the synthesis of RNA interfering (RNAi) molecules that will interact with Th2 cytokine mRNA in cancer cells that inhibit the synthesis of the cytokines.

It is hypothesized that the use of either cytokine antagonists, together with CpG ODNs, will be an effective treatment against drug-resistant and -sensitive tumors that will restore the Th1/Th2 balance and reactivate the patient's adaptive antitumor immunity. Recent reports have shown that the above anticancer technologies may be developed as effective anticancer therapies.

1) IL-4 delta 2 splice variant is an antagonist of IL-4 activity by binding to IL-4 receptors. IL-4 is a 15 kDa glycoprotein secreted by activated Th2 cells and $Fc\varepsilon RI^{+}$ hematopoietic

cells (mast cells, basophils, monocytes and DCs). The Th2 cytokine IL-4 is involved in the induction of allergies and with the development of AIDS in HIV-1-infected people (20, 58). The enhanced release of IL-4 from hematopoietic cells is paralleled by increased IgE synthesis by B cells, leading to the inhibition of Th1 cell activities in patients with tumors belonging to subgroups A and B (Table I).

It was reported, by Alms *et al.* (78), that the IL-4 gene is expressed in T cells into IL-4 full-length mRNA and a splice variant of IL-4 mRNA lacking exon 2. These mRNAs are translated to IL-4 and IL-4 delta 2 protein molecules, respectively. The splice variant IL-4 delta 2 is also expressed by B lymphoid cells (79). Activated T cells treated with IL-4 delta 2 were inhibited since the latter occupied the cell's IL-4 R alpha (80). Monocytes and B cells were also inhibited by the IL-4 antagonists (81). Excess antagonist IL-4 delta 2 competes with IL-4 and binds to the IL-4 alpha receptor on monocytes, T and B cells and prevents binding of IL-4 and the induction of the cells to express the IL-4-responsive genes by $CD4^+$ T cells and prevents the IL-4-induced switch to the synthesis of IgE by B cells. Individuals (about 10% of the human population) who have higher levels of IL-4 delta 2 than IL-4 in the blood were reported to be able to prevent the development of tuberculosis (TB) after infection with *Mycobacterium tuberculosis* (82).

2) IL-6 delta 3 protein, a splice variant of IL-6 protein, is the antagonist of IL-6. Human tumors (subgroup C, Table I) synthesize and release the Th2 cytokine IL-10, the inhibitor of IFN-alpha and -beta synthesis by PDCs, inhibiting the cellular arm of the adaptive immune response, the activation of CTL precursors to antitumor CTLs. The synthesis of IL-10 by the tumor cells is induced by the release of IL-6 by tumor-infiltrating macrophages. Therefore, since inhibition of IL-10 with an IL-10 antagonist is not yet available, it may be possible to inhibit IL-6 synthesis by infiltrating macrophages with the splice variant of IL-6 delta 3.

Alberti *et al.* (83) reported that full-length IL-6 mRNA and IL-6 delta 5 mRNA are synthesized by cultured human peripheral blood mononuclear cells and by RCC cells. The IL-6 splice variant protein binds to IL-6R and acts as an IL-6R antagonist. Yatsenko *et al.* (84) reported that three isoforms of IL-6 mRNA were synthesized in mouse bone marrow and spleen cells: full-length IL-6, IL-6 delta 3 and IL-6 delta 5. Yang *et al.* (85) used computer-aided design based on the crystal structure of human IL-6 (hIL-6) and its receptor, hIL-6R, to synthesize a novel human IL-6 antagonistic peptide (PT). It was reported that PT possessed a very high affinity to hIL-6R and offered a practical means of imposing a long-term blockade of IL-6 activity *in vitro*. PT acted as an excellent antagonist of growth arrest and apoptosis in a murine M1 myeloid cell line, when induced by hIL-6.

3) Antibodies to IL-6R are IL-6 antagonists. Nishimoto and Kishimoto (86) developed humanized antibodies to the IL-6 receptor (IL-6R) that are able to block the binding of IL-6 molecules to IL-6 receptors and reported that molecular therapy targeting of IL-6 has been shown in clinical trials to be therapeutically effective in inflammatory disease such as rheumatoid arthritis. Clinical trials with the humanized IL-6 antibodies in the treatment of multiple myeloma patients were also conducted. The administration of anti-IL-6R antibodies to patients with arthritis and multiple myeloma greatly improved the patients' condition for several weeks, but the symptoms of the disease returned.

(a) IL-6 mutant protein as an IL-6 antagonist: Brakenhoff *et al.* (87) reported that neutralizing antibodies specific to IL-6 bind two distinct sites of the IL-6 protein molecule: site I is a receptor-binding site on the 80-kDa IL-6R, and site II is involved with the binding of gp130, the signal inducer. The authors mutated IL-6 site II with a double mutation Gln-160 with Glu (Q160E) and Thr-163 with Pro (T163P), and reported that IL-6 mutant protein with a mutation in site II could bind to the 80-kDa hIL-6R, and antagonized the biological activity by preventing the binding of wild-type hIL-6 to HepG2 cell IL-6R. Renné *et al.* (88) constructed fusion proteins that consisted of the soluble form of hIL-6R covalently linked to IL-6 receptor antagonists. These fusion proteins directly bind to gp130, the signal transducer. At concentrations of 10-50 nM, the IL-6 antagoninsts completely neutralized the IL-6 biological activity. As IL-6 type cytokines, including IL-11 and additional family members, the fusion proteins prevent the activity of cytokines that act *via* gp130 homodimers. The authors suggested, that due to targeting of gp130, these fusion proteins: "might be useful therapeutic tools in disease states that are related to IL-6 family".

The above reports on the availability of a number of different IL-6 antagonists provide the basis for including these IL-6 inhibitors in the treatment of cancer patients, especially by injection into melanoma tumor microenvironments.

4) RNA interference of IL-10 in leukemia B1 tumor cells. McCarthy *et al.* (89) reported that RNA interference (RNAi) to IL-10 mRNA was designed, modeled to a hairpin configuration, and was used to treat chronic lymphocytic leukemia (CLL). A malignant B1 cell line (LNC), derived from an NZB mouse (a murine model for CLL), was used as a target for IL-10 RNAi. The authors reported that IL-10 RNAi lowered IL-10 protein synthesis by the treated cells. At 2 μM IL-10 RNAi, a G2/M cellular block was initiated, mRNA levels for the M-phase inducer phosphatase cdc25c decreased and apoptosis was induced in the tumor cells. These studies suggested that blocking IL-10 is of potential value for treating certain leukemias, either alone or in combination therapy.

It seems logical to suggest that the use of the RNAi technique to inhibit IL-4 and IL-6 synthesis in cancer patients may become a useful method for treating human leukemias.

5) IFN-alpha and -beta are immunotherapeutic proteins for treatment of cancer patients.

(i) PDCs, the producers of type I IFN, are inhibited by tumors. IFN-alpha and -beta are produced by human cell cultures and are used as immunotherapeutic proteins for treatment of cancer patients (90). However, long-term treatment with large doses of exogenous interferon preparations may cause an autoimmune response in some patients. It was also reported that human PDCs are the major producers of type I IFNs in the human organism. These cells express Toll-like receptor 9 (TLR9) that serves as a receptor for unmethylated bacterial DNA fragments, which induce the PDCs to release *in vivo* large amounts of type I IFNs. Treatment of cancer patients with bacterial-like unmethylated synthetic CpG ODNs also induced PDCs to release IFN-alpha and -beta, the inhibitors of the synthesis of Th2 cytokines by $CD4^+$ T cells, and induce IL-4-inhibited Th1 cells to synthesize IL-2, IL-12 and IFN-gamma that activate the $CD8^+$ cell precursors to differentiate into CTLs, the antitumor adaptive immune response (91).

Vicari *et al.* (92) investigated the phenotype and functions of tumor infiltrating DCs in mouse tumor models, and reported that the DCs were immature and paralyzed. Treatment of tumor-bearing mice with CpG ODN, together with anti-IL-10R antibody, triggered activation and IL-12 production in normal DCs and elicited antitumor immune memory cells.

Hartmann *et al.* (93) studied the presence and function of PDCs in human solid tumors and reported that they infiltrated the tumor tissue in patients with HNSCC. The tumors diminished the PDCs' ability to produce IFN-alpha in response to CpG ODNs, since the tumor induced the down-regulation of TLR9, the CpG ODN receptor. However, in tumor-draining lymph nodes the suppression of the CpG ODN induction to release PDC IFN-alpha was less pronounced. Kemp *et al.* (94) tested the effect of CpG ODN on blood PDCs and reported that PDCs are the cells that produce large amounts of type I IFNs, inducers of TNF-related antitumor apoptosis response.

The increase of Th2 cytokines IL-4 and IL-10 by HNSCC tumor cells (Table IA) resulted in the down-regulation of the TLR9 CpG ODN receptors on PDCs that infiltrate into solid tumors. Therefore, systemic injection of CpG ODNs to cancer patients may induce PDCs in the blood and in unaffected lymph nodes to release large amounts of IFN-alpha and -beta, with the potential to inhibit the increase of the Th2 cytokines IL-4 and IL-10. In addition, type I IFNs stimulate IL-4-inhibited Th1 cells to express IL-2 and IL-12, that allow the induction of $CD8^+$ CTL precursors to become antitumor CTLs and also induce B cells to synthesize antitumor IgG antibodies. Indeed, Nakamori *et al.* (95) reported that IL-12 and IL-18 immune gene therapy against mouse colon cancer promoted the generation of IFN-gamma production by Th1 cells with therapeutic efficacy, reduction of tumor size and absence of metastases.

Kawarada *et al.* (96) investigated the therapeutic effect of CpG ODN on established mouse tumors AG104A, IE7 fibrosarcoma, B16 melanoma and 3LL lung carcinoma. The authors reported that repeated peritumoral injection of CpG ODN caused complete rejection, or strong inhibition, of tumor growth, while systemic application of CpG ODN had only a partial effect. The tumor rejection was mediated by NK cells and antitumor $CD8^+$ CTLs. Heckelsmiller *et al.* (97) reported that weekly injections of CpG ODN into the margin of established tumors of syngeneic C26 tumor cells or Renca kidney cancer cells in BALB/c mice resulted in regression of tumors and complete cure of mice, to which tumor-specific $CD8^+$ T cells and innate effector cells contributed. The authors concluded that peritumor CpG ODN monotherapy elicits a strong $CD8^+$ T cell response and innate effector mechanisms that act to overcome the unresponsiveness of the immune system toward a growing tumor.

Lonsdorf *et al.* (98) studied the protective antitumor response, using a murine RMA lymphoma in a C57BL/6 mouse model, to test the effect of injection of CpG ODNs directly into the tumor. It was reported that complete remission was achieved in immune-competent mice, but not in T cell/B cell-deficient RAG-1 knockout mice. The authors determined that the tumor-specific $CD4^+$ and $CD8^+$ T cell responses of the type 1 effector class were responsible for tumor rejection, and that adoptive transfer of these cells to RAG-1 knockout mice protected against the RMA lymphoma.

Hafner *et al.* (99) studied the antimetastatic effects of CpG-containing DNA in a mouse model of experimental metastatic syngeneic fibrosarcoma or thymoma cells that were injected into 4 mice strains. CpG ODN was injected *i.v.* before tumor cell application and it was reported that CpG ODN strongly inhibited tumor metastases and a high level of type I IFN was found in the mouse sera.

Carpentier *et al.* (100) evaluated the ability of CpG ODNs to cause rejection of implanted syngeneic neuroblastomas cell line (neuro2a) by the subcutaneous (*s.c.*) route in A/J mice. Daily injections of 10 mg CpG ODNs for 15 days led to the eradication of 5-mm-diameter tumors in one-half of the animals and significant tumor growth inhibition (88%) as compared with the controls. The authors concluded that immunostimulatory CpG ODNs may induce the rejection of established tumors. In a subsequent study, Carpentier *et al.* (101) inoculated Lewis rats intracerebrally with syngeneic CNS-1 glioma cells and, 5 days later, injected CpG ODNs into the tumor bed. Of

8 rats, 88% of the animals survived more than 90 days, while the 14 untreated controls died within 23 days. The cured animals were protected against a second tumor challenge. Injection of CNS-1 tumor to nude mice by the *s.c.* route, followed by injection of CpG ODNs, had no effect on tumor progression, suggesting that CpG ODNs increased the tumor infiltration with macrophage/microglial cells, $CD8^+$ T cells and NK lymphocytes. The authors concluded that treatment of malignant glioma patients with CpG ODNs, who exhibit depressed *in vivo* reactivity of peripheral blood and tumor-infiltrating lymphocytes, could lead to glioma rejection, a new immunotherapeutic approach.

Brunner *et al.* (102) reported that DCs manipulated *ex vivo*-induced tumor immunity in an experimental murine tumor model and studied whether DC maturation affects the T cell-activating potential *in vitro* and induction of tumor immunity *in vivo*. CpG ODN-1826 stimulated the maturation of DCs, that was in correlation with the secretion of IL-12 and induction of T cell proliferation. Flow cytometric analysis of costimulatory molecules and MHC class II showed that DC maturation was stimulated most by ODN-1862. BALB/c mice harboring rapidly growing colon carcinoma tumors were administered with CpG ODN 3 days after tumor challenge. Stimulated DCs that were co-cultured with irradiated tumor cells induced prophylactic protection most effectively. The authors suggested that CpG ODN-enhanced DC maturation may represent an efficient means to improve clinical tumor vaccination.

Ballas *et al.* (103) reported that specific motifs of CpG ODNs vary in their ability to induce antitumor activities in different tumor models. CpG ODN (1585), which has a chimeric backbone in combination with poly(G), is a potent inducer of NK lytic activity and can induce regression of established melanoma tumors in mice requiring the presence of NK cells. In contrast, CpG ODN (1826), which was optimized for activation of B cells and Th1-like cytokine expression, had no therapeutic effects on melanoma, but was effective at inducing regression of EL4 murine lymphoma. The authors concluded that CpG ODN immunotherapy requires careful analysis of the cellular properties of the various CpG motifs.

6) Treatment with CpG ODNs enhances the efficacy of monoclonal antibody therapy of lymphoma. Wooldridge *et al.* (104) compared the efficiency of anticancer treatment with antitumor monoclonal antibodies alone or together with CpG ODN. It was noted that a single dose of CpG ODN appeared to be as effective as multiple doses of IL-2 at inhibiting tumor growth when combined with antitumor monoclonal antibodies. Ninety percent of immunocompetent C3H mice that were inoculated with 2,500 tumor cells *i.p.* and treated with monoclonal antibody developed tumors, while only 20% of mice that were treated with the monoclonal antibody and CpG ODN developed tumors.

Van Ojik *et al.* (105) used murine cancer models to stimulate different effector cells to explore the combined ability of antitumor monoclonal antibodies and CpG ODNs A or B in immunotherapy of 38C13 syngeneic murine lymphoma in C3H mice. The authors reported that CpG ODNs A and B enhanced the efficacy of the antitumor antibodies. Heckelsmiller *et al.* (106) reported that either mature antigen-pulsed DCs or peritumoral injections of CpG ODN were effective for treatment of small, established tumors, but were ineffective against large established tumors (1 cm diameter) in a syngeneic murine colon carcinoma. Co-injection of CpG ODN together with mature, antigen-pulsed DCs caused rejection of large tumors, leading to a long-term cure of the cancer-bearing mice.

These experimental results clearly demonstrated that injection of CpG ODN into the periphery of mouse solid tumors *in vivo,* or into the tumors in mice combined with antitumor monoclonal antibodies, achieved cure of the tumors.

7) Yogurt feeding of mice inhibits promotion and progression of experimental colorectal cancer. Perdigon *et al.* (107) studied the effect of yogurt feeding on BALB/c mice which developed colorectal carcinoma after treatment with the carcinogen 1,2 dimethylhydrazine (DMH). Yogurt was added to the diet for 10 consecutive days, with the procedure repeated every 10 days for 6 months. The authors reported that yogurt feeding increased the number of apoptotic tumor cells and induced IFN-gamma and TNF-alpha that were regulated by an increase in IL-10. In a subsequent study (108), de Moreno de LeBlanc *et al.* reported that mice treated with the carcinogen DMH and receiving yogurt cyclically after DMH treatment did not develop tumors (DMH-yogurt group). There was no correlation between a high level of IL-10 and the regulatory T cell population, while IL-4 and apoptotic cells increased in the yogurt-DMH mouse group that received yogurt only 10 days before DMH. The authors concluded that feeding yogurt to carcinogen-treated mice inhibited tumor progression and promotion by stimulating cellular apoptosis.

Yogurt contains viable lactic bacteria, *Lactobacillus delbrueckii* and *Streptococcus thermophilus,* at a density of 10^8 cells/ml. These bacterial cells are present in the human intestine and, after disintegration, the unmethylated bacterial DNA is released and degraded in the gut. TLR9-positive PDCs bind the bacterial DNA fragments and release large amounts of IFN-alpha and -beta, inhibitors of Th2 cytokine synthesis by Th2 cells, activators of Th1 cell cytokine synthesis, and also activate antitumor CTLs. Hence, continued consumption of yogurt by humans may reduce the incidence of colon cancer. It is of interest that Sudo *et al.* (109) reported that dietary nucleic acid and

intestinal microbiota synergistically promoted a shift in the Th1/Th2 balance toward Th1-skewed immunity that induced the development of antitumor CTLs.

E. Discussion, Hypothesis and Implications

a. Discussion

The present review analyzed the published reports on three aspects of human cancer: (i) the skewing of the cancer patients' Th1/Th2 balance toward Th2 cytokines and the use of the patients' serum for monitoring the Th2 cytokine levels, which are the bio- indicators of the existence of a tumor (provided that the individual does not have an allergy); (ii) the ability of tumor cells to release IL-10 into the tumor microenvironment, inhibiting the infiltrating innate and adaptive immune cells; and (iii) new approaches to reactivate the inhibited adaptive immunity of cancer patients by treatment with cytokine antagonists combined with CpG ODNs. This combination biotherapy is designed to inhibit the activity of the Th2 cytokines IL-4 and IL-6 by blocking their receptors on $CD4^+$ T cells, B cells and macrophages, respectively. The combined treatment with CpG ODN enhances the release of type I IFNs, inducers of Th1 cell synthesis, and release of IL-2, IL-12 and IFN-gamma, leading to the activation of CTL precursors to become antitumor CTLs.

(i) Determination of Th2/Th1 cytokines in the blood of healthy human populations to identify the tumor-bearing individuals. Reports on the levels of Th1 and Th2 cytokines have indicated that all human tumors studied in different tissues of individuals have a common effect on the Th1/Th2 balance, causing a marked increase of Th2 cytokines (Table I and Figure 3A) compared to healthy individuals (Figure 2). The authors of these reports suggested that a marked increase in IL-4, IgE, sCD23, IL-10 and IL-6 should be considered as cancer biomarkers, which allow early detection of the existence of a tumor in individuals in whom a high level of Th2 cytokines has been detected. Since increases of IgE and Th2 cytokine levels were reported in inflammations caused by allergens or infection with pathogenic microorganisms, it will be necessary to develop a blood test that will ensure that the tested individuals are not suffering from allergy or microbial infection or suffering from a genetic disorder that skews the Th1/Th2 cytokine balance toward Th2 cytokines.

(ii) The role of tumors in the inhibition of the innate and adaptive immune cells in the tumor microenvironment. IL-10 is one of the Th2 cytokines which inhibit the cancer patients' adaptive immune response and the ability of PDCs to release type I IFNs. Therefore, the ability of tumors to synthesize and release IL-10 into the tumor microenvironment is designed to inhibit the tumor-infiltrating leukocytes such as DCs and PDCs. The reported studies showed that tumor-infiltrating macrophages that synthesize and release the Th2 cytokine IL-6 locally induce the expression of the IL-10 gene and the release of the IL-10 cytokine by the tumor cells. Therefore, the combined Th2 cytokine increase in the patients' blood and the tumor local production of IL-6 and IL-10 are additive and lead to the collapse of the antitumor adaptive immune response, allowing tumor progression without interference by the adaptive immune system.

b. Cancer biotherapy: new approaches to reactivate the inhibited anticancer adaptive immunity of cancer patients – a hypothesis.

The experimental data that was reviewed above led to the conclusion that tumor cells either release allergen-like glycoproteins early in the tumor development or that systemically skew the Th1/Th2 cytokine balance toward Th2 cytokine dominance (Figure 3A). The Th2 cytokine IL-4 inhibits Th1 cytokine synthesis and induces B cells to stop IgG synthesis and switch to IgE production. With the gradual decline of the patient's adaptive immune response, the tumor increases in mass and releases cells that migrate to lymph nodes and other organs, forming metastatic tumors. It is possible to assume that the tumor cells are responsible for the destruction of the host's protective immune response. The logical conclusion may be that skewing of the Th1/Th2 cytokine balance may be prevented by inhibiting the biological activity of IL-4, IL-6 and IL-10 by blocking IL-4R and IL-6R by the respective cytokine antagonists IL-4 delta 2 and IL-6 delta3. In the absence of the Th2 cytokines, the polarized Th1 cells will produce IL-2, IL-12 and IFN-gamma, and the antitumor CTLs will eliminate the tumor cells. Figure 3B presents a hypothesis suggesting that individuals who produce more of the IL-4 antagonist IL-4 delta 2 than IL-4 may be resistant to tumors. Therefore, testing the natural levels of IL-4 delta 2 in human serum is needed to determine the presence of tumor-resistant individuals in population studies (Figure 4A). Similarly, testing for the level of IL-6 delta 3 in the serum may provide information on tumor resistance.

It is also hypothesized (Figure 4B) that injection of the IL-6 antagonist IL-6 delta 3 into the tumor microenvironment will block IL-6 receptors (IL-6R) on the tumor cells and will prevent the induction of IL-10 synthesis and release.

A new approach to prevent the skewing of the Th1/Th2 cytokine balance toward Th2 cytokines by tumor cells was presented in recent experimental studies, reporting that CpG ODNs which bind to $TLR9^+$ PDCs induce the release of large amounts of type I IFNs alpha and beta. The IFNs inhibit the release of Th2 cytokines from the hematopoietic cells, induce B cells to switch from IgE to IgG synthesis and induce Th1 cells to release cytokines that activate the CTL precursors to antitumor CTLs.

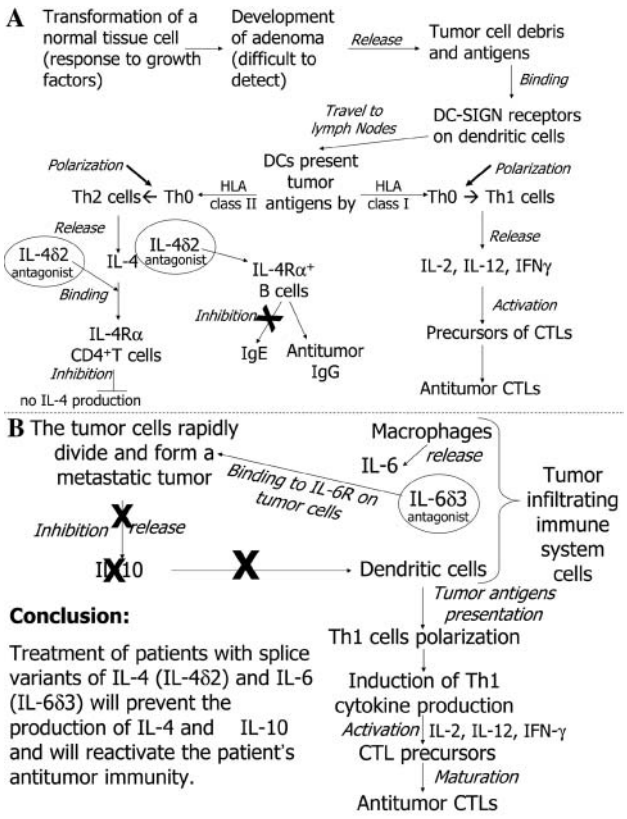

Figure 4. *The molecular-immunological basis for treatment of cancer patients with the cytokine antagonists IL-4 delta 2 (A) and IL-6 delta 3 (B). A hypothesis.*

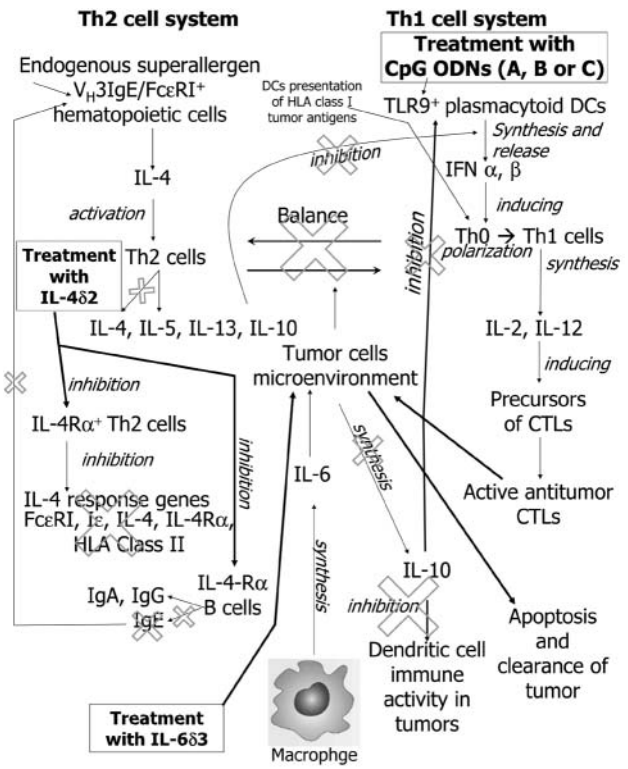

Figure 5. *Biotherapy of human cancers with a combination of cytokine antagonists of IL-4 and IL-6 and interferon type I inducer CpG ODN – a proposal.*

c. Implications for cancer diagnosis and treatment.

The analysis of the experimental data reviewed above leads to the following conclusions:

i) The adaptive immune response in tumor-bearing individuals is inhibited long before a tumor can be diagnosed by conventional methods. Therefore, routine blood tests to determine the Th1/Th2 cytokines in the serum of healthy and diseased individuals needs to be carried out to determine the levels of the Th2 cytokines IL-4, IL-5, IL-6 and the levels of Th1 cytokines IL-2, IL-12 and IFN-alpha, IFN-beta and IFN-gamma.

ii) Individuals who display increased Th2 cytokine levels in the serum should be tested for increased IgE and sCD23 serum levels. The presence of allergy or microbial infection must be ruled out.

iii) At an early stage of tumor development, biotherapy treatments should include IL-4 and IL-6 antagonists (after determination of their efficacy in experimental models of cancers). The treatment of tumor-bearing individuals should be with cytokine antagonists combined with CpG ODNs (Figure 5).

iv) In individuals who had been diagnosed at a later stage of tumor development, the levels of the serum Th2 cytokines will determine the amounts of the cytokine antagonists required for biotherapy. Chemotherapy treatment could be used to reduce the tumor mass.

F. Conclusion

The current use of chemotherapy and X-irradiation to reduce tumor mass in cancer patients has led to improvements in the health of cancer patients and remission and even complete cure of different types of tumors. However, these techniques did not take into consideration that the clearance of a tumor depends on the patients' adaptive immune response. The understanding of the molecular and immunological mechanisms of the innate and adaptive immune system cells led to the search for Th2 cytokine antagonists that counteract the increase of Th2 cytokines by tumor cells. Reactivation of the anticancer adaptive immunity by using anti-Th2 cytokine monoclonal antibodies in combination with CpG ODNs and Th2 cytokine antagonists in combination with CpG ODNs will provide new

avenues for anticancer treatments, with the potential to develop curative antiviral drugs for cancer biotherapy. The biotherapy approach aims to protect and reactivate the patients' adaptive immunity against the tumor cells.

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References

1 Hajdu SI: 2000 years of chemotherapy of tumors. Cancer *103*: 1097-1102, 2005.
2 Hanahan D and Weinberg RA: The hallmarks of cancer. Cell *100*: 57-79, 2000.
3 Longley DB and Johnston PG: Molecular mechanisms of drug resistance. J Pathol *205*: 275-292, 2005.
4 Ardavin C: Origin, precursors and differentiation of mouse dendritic cells. Nature Rev Immunol *3*: 1-9, 2003.
5 Gordon S: Alternative activation of macrophages. Nature Rev Immunol *3*: 23-35, 2003.
6 Becker Y: Immunological and regulatory functions of uninfected and virus-infected immature and mature subtypes of dendritic cells – a review. Virus Genes *26*: 119-139, 2003.
7 Iwasaki A and Medzhitou R: Toll-like receptor control of the adaptive immune responses. Nature Rev Immunol *5*: 987-995, 2004.
8 Kawakami T and Galli SJ: Regulation of mast-cell and basophil function and survival by IgE. Nature Rev Immunol *3*: 773-786, 2002.
9 Hayday H and Tigellar R: Immunoregulation in the tissues by gamma-delta T cells. Nature Rev Immunol *3*: 233-242, 2003.
10 Ohlin M and Zouali M: The human antibody repertoire to infectious agents: implications for disease pathogenesis. Mol Immunol *40*: 1-11, 2003.
11 Langenkamp A, Messi M, Lanzavecchia A and Sallusto F: Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells. Nature Immunol *1*: 311-316, 2000.
12 Bousso P and Robey E: Dynamics of $CD8^+$ T cell priming by dendritic cells in intact lymph nodes. Nature Immunol *4*: 579-585, 2003.
13 Miller MJ, Safrince O, Parker I and Cahalan MD: Imaging the single cell dynamics of $CD4^+$ T cell activation by dendritic cells in lymph nodes. J Exp Med *200*: 847-856, 2004.
14 Granucci F, Zanoni I, Feau S, Capuano G, Ricciardi C and Gastognoli P: The regulatory role of dendritic cells in the immune response. Arch Allergy Immunol *134*: 179-185, 2004.
15 Mempel TR, Henrickson SE and von Andrian UH: T-cell priming by dendritic cells in lymph nodes occurs in three distrint phases. Nature *427*: 154-159, 2004.
16 Friedl P and Brocker EB: TCR triggering on the move: diversity of T-cell interactions with antigen presenting cells. Immunolog Rev *186*: 83-89, 2002.
17 Moody DB and Porcelli SA: Intracellular pathways of CD1 antigen presentation. Nature Rev Immunol *3*: 11-22, 2003.
18 Mosmann TR and Coffman RL: TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Ann Rev Immunol *7*: 145-173, 1989.
19 Kidd P: Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. Altern Med Rev *8*: 223-246, 2003.
20 Becker Y. CpG ODN treatments of HIV-1-infected patients may cause the decline of transmission in high risk populations – a review, hypothesis and implications. Virus Genes *30*: 251-266, 2005.
21 Becker Y: The molecular mechanism of human resistance to HIV-1 infection in persistently infected individuals – a review, hypothesis and implications. Virus Genes *31*: 1, 2005.
22 Demissie A, Owiafe P, Jeffries D, Hill P, Rook GAW, Zumla A, Weldingh K, Brock I, Andersen P, Deherty TM and the VACSEL study group: Healthy individuals that control a latent infection with *Mycobacterium tuberculosis* express high levels of Th1 cytokines and the IL-4 antagonist IL-4 delta 2. Immunology *112*: 669-673, 2004.
23 Etzioni R, Urban N, Ramsey S, McIntosh M, Schwartz S, Reid B, Radich J, Anderson G and Hartwell L: The case for early detection. Nature Rev Cancer *3*: 1-10, 2003.
24 Hallgren R, Arrendal H, Hiesche K, Lunquist G, Nou E and Zetterstrom O: Elevated serum immunoglobulin E in bronchial carcinoma: its relation to histopathological prognosis of the cancer. J Allergy Clin Immunol *67*: 398-406, 1981.
25 Ownby DR, Ownby HE, Roi LD, Howard LM, Heppner GH and Brennan MJ: Prognostic significance of serum IgE levels in primary breast cancer. Breast Cancer Res Treat *26*: 221-226, 1982.
26 Mosseri R, Finkelstein Y, Monselize Y and Garty BZ: Large T-cell lymphoma in a 13-yaer-old girl with hyperimmunoglobulinemia E syndrome. Pediatric Allergy Immunol *13*: 143-146, 2002.
27 Chechimi J, Elder M, Greene J, Noroski L, Stiehm R, Winkelstein JA and Sullivan KE: Cytokine and chemokine dysregulation in hyper IgE syndrome. Clin Immunol *100*: 49-56, 2001.
28 Del Prete G, Tiri A, Maggi E, De Carli M, Macchia D, Passonchi P, Rossi ME, Peitrogrande ML, Rici M and Romagnani S: Defective *in vitro* production of γ-interferon and tumor necrosis factor-α by circulating T cells from patients with hyperimmunoglobulin E. J Clin Invest *84*: 1830-1835, 1989.
29 Borges WG, Augustine NH and Hill HB: Defective interleukin 12/interferon-gamma pathway in patients with hyperimmunoglobulinemia E syndrome. J Pediatr *136*: 176-180, 2000.
30 Shirafreji Y, Matsuura H, Sato A, Kauzaki H, Katayama H and Arata J: Hyperimmunoglobulin E syndrome: a sign of TH1/TH2 imbalance? Eur J Dermatol *9*: 129-131, 1999.
31 Netea MG, Schneerberger PM, de Vries E, van der Meer JW and Koolen ML: Th1/Th2 cytokine imbalance in a family with hyper-IgE syndrome. Neth J Med *69*: 349-353, 2002.
32 Molica S, Levato D, Dell'Olio M, Matera R, Minervini M, Dattilo A, Carotenuto M and Musto P: Cellular expression and serum circulating levels of CD23 in B-cell chronic lymphocytic leukemia. Implications for prognosis. Hematological *81*: 428-433, 1996.
33 Sarfati M, Chevret S, Chastang C, Biron G, Stryckmans P, Delespesse G, Binet J-L, Merle-Beral H and Bron D: Prognostic importance of serum-soluble CD23 level in chronic lymphocytic leukemia. Blood *88*: 4259-4264, 1996.

34 Schroeder JR, Saah AJ, Hoover DR, Margolick JB, Ambinder RF, Martinez-Maza O, Breen EC, Jacobson LP, Variakojis D, Rowe DT and Armenian HK: Serum-soluble CD23 level correlates with subsequent development of AIDS-related non-Hodgkin's lymphoma. Cancer Epidem Biomark Prevent *8*: 979-984, 1999.

35 Schroeder JR, Saah AJ, Ambinder RF, Martinez-Maza O, Breen EC, Variakojis D, Margolick JB, Jacobson LP, Rowe DT and Hoover DR: Serum sCD23 level in patients with AIDS-related non-Hodgkin's lymphoma is associated with the absence of Epstein-Barr virus in tumor tissue. Clin Immunol *93*: 239-244, 1999.

36 Pellegrini P, Berghella AM, Del Berto T, Cicia S, Adorno D and Casciani CU: Disregulation in TH1 and TH2 subsets of $CD4^+$ T cells in peripheral blood of colorectal cancer patients and involvement in cancer establishment and progression. Cancer Immunol Immunother *42*: 1-6, 1996.

37 Sparano A, Lathers DMR, Achille N, Petruzzelli GJ and Young MRI: Modulation of Th1 and Th2 cytokine profiles and their association with advanced head and neck squamous cell carcinoma. Otolaringol Head Neck Surg *131*: 573-576, 2004.

38 Yamazaki K, Yamo T, Kameyama T, Suemitsu R, Yoshino I and Sugio K: Clinical significance of serum TH/1TH2 cytokines in patients with pulmonary adenoma. Surgery *131*: S236-241, 2002.

39 Ito N, Nakamura H, Tanaka Y and Ohgi S: Lung carcinoma: analysis of T helper type 1 and 2 cells and T cytotoxic type 1 and 2 cells by intracellular cytokine detection with flow cytometry. Cancer *85*: 2359-2367, 1999.

40 Goedegbuure PS, Douville CC, Doherty JM, Linehan DC, Lea K-Y, Gangly EK and Eberlein TJ: Simultaneous production of T helper 1-like cytokines and cytolytic activity by tumor-specific T cells in ovarian and breast cancers. Cell Immunol *175*: 150-156, 1997.

41 Kozlowski L, Zakrzewska I, Tokajuk P and Wojtukiewicz MZ: Concentrations of interleukin-6 (IL-6), interleukin-8 (IL-8) and interleukin-10 (IL-10) in blood serum of breast cancer patients. Rocz Akad Med Bialymst *48*: 82-84, 2003.

42 Filella X, Alcover J, Zarco MA, Beardo P, Molina R and Ballesta AM: Analysis of type T1 and T2 cytokines in patients with prostate cancer. Prostate *44*: 271-274, 2000.

43 Mori T, Takada R, Watanabe R, Okamato S and Ikeda Y: T-helper Th/1Th2 imbalance in patients with previously untreated B-cell diffuse large cell lymphoma. Cancer Immunol Immunother *50*: 566-568, 2001.

44 Podhorecka M, Dmoszynska A, Rolinski J and Wasik E: T type 1/type 2 subset balance in B-cell chronic lymphocytic leukemia – the three-color flow cytometry analysis. Leukemia Res *26*: 657-660, 2002.

45 Fujisao S and Tsuda H: Th1/Th2 balance alteration in the clinical course of a patient with pure red cell aplasia and thymoma. Br J Haematol *103*: 308-310, 1998.

46 Dummer W, Becker JG, Schwaaf A, Leverkus M, Moll T and Brocker EB: Elevated serum levels of interleukin-10 in patients with metastatic malignant melanoma. Melanoma Res *5*: 67-68, 1995.

47 Nemunaitis J, Fong T, Shabe P, Martineau D and Ando D: Comparison of serum interleukin-10 (IL-10) levels between normal volunteers and patients with advanced melanoma. Cancer Invest *19*: 239-247, 2001.

48 Lauerova L, Dusek L, Simickova M, Kocak I, Vagundova M, Zaloudik J and Kovarik J: Malignant melanoma associates with Th1/Th2 imbalance that coincides with disease progression and immunotherapy response. Neoplasma *49*: 159-166, 2002.

49 De Vita F, Orditura M, Galizia G, Romano C, Roscigno A, Lieto E and Catalano G: Serum interleukin-10 levels as a prognostic factor in advanced non-small cell lung cancer patients. Chest *117*: 365-373, 2000.

50 Hatanaka H, Abe Y, Kamiya T, Morino F, Nagata J, Tokunaga T, Shika Y, Suemizu H, Kaijima H, Tsuchida T, Yamazaki H, Inoue H, Nakamura M and Ueyama T: Clinical implications of interleukin (IL)-10 induced by non-small cell lung cancer. Ann Oncol *11*: 815-819, 2000.

51 Neuner A, Schindel M, Wildenberg U, Muley T, Lahm H and Fischer JR: Prognostic significance of cytokine modulation in non-small cell lung cancer. Int J Cancer *101*: 287-292, 2002.

52 Wittke F, Hoffman R, Buer J, Dallmann I, Oevermann K, Sel S, Wandert T, Ganser A and Atzpodien J: Interleukin 10-(IL-10): an immunosuppressive factor and independent predictor in patients with metastatic renal cell carcinoma. Br J Cancer *79*: 1182-1184, 1999.

53 Negrier S, Perol D, Menetrier-Caux C, Pallardy M, Douillard JY, Chevreau C, Lasset C, Blay JY and Groupe Francais d'Immunotherapy: Interleukin-6, interleukin-10 and vascular endothelial growth factor in metastatic renal cell carcinoma: prognostic value of interleukin-6 from the Groupe Francais d-Immunotherapy. J Clin Oncol *22*: 2371-2378, 2004.

54 Berghella AM, Pellegrini P, Del Beato T, Adorno D and Casciani CU: IL-10 and sIL-2R serum levels as possible peripheral blood prognostic markers in the passage from adenoma to colorectal cancer. Cancer Biother Radiopharm *12*: 265-272, 1997.

55 Lissoni P, Merlini D, Pirato D and Meregalli S: Interleukin-4 blood concentrations in early and metastatic human solid neoplasms. Int J Biol Markers *12*: 75-78, 1997.

56 Kim J, Modlin RL, Moy RL, Dubinett SM, McHugh T, Nickdoff BJ and Uyemura K: IL-10 production in cutaneous basal and squamous cell carcinoma. J Immunol *155*: 2240-2247, 1995.

57 Mor G, Visintin I, Lai Y, Zhao H, Schwartz P, Rutherford T, Yue L, Bary-Ward P and Ward DC: Serum protein markers for early detection of ovarian cancer. Proc Natl Acad Sci *102*: 7677-7682, 2005.

58 Becker Y: Allergen-like gp120 molecules from HIV account for AIDS. ASM News *70*: 565-570, 2004.

59 Satthaporn S, Robins A, Vassanasiri W, El-Sheemy M, Jibril JA, Clark D, Valero D and Eremin O: Dendritic cells are dysfunctional in patients with operable breast cancer. Cancer Immunol Immunother *53*: 510-518, 2004.

60 Singer CF, Kubista E, Garmondi R and Cullen KJ: Local feedback mechanisms in human breast cancer. Breast Cancer Res Ther *63*: 95-104, 2000.

61 Crichton MB, Nichols JE, Zhao Y, Bulum SE and Simpson ER: Expression of transcripts of interleukin-6 and related cytokines by human breast tumors, breast cancer cells and adipose stromal cells. Mol Cell Endocrinol *118*: 215-220, 1996.

62 Jablonska E and Pietruska Z: Changes in soluble IL-6 receptor and IL-6 production by polymorphonuclear cells and whole blood cells of breast cancer patients. Arch Immunol Ther Exp (Warsz) *46*: 25-29, 1998.

63 Blais Y, Gringras S, Haagensen D.E, Labrie F and Simard J: Interleukin-4 and interleukin-13 inhibit estrogen-induced breast cancer cell proliferation and stimulate GCDFP-15 expression in human breast cancer cells. Mol Cell Endocrinol *121*: 11-18, 1996.

64 Vermi W, Bonecchi R, Faccetti F, Bianchi D, Sozzani S, Festa S, Berenzi A, Cella M and Colonua M: Recruitment of immature plasmacytoid dendritic cells (plasmacytoid monocytes) and myeloid dendritic cells in primary cutaneous melanomas. J Pathol *200*: 255-268, 2003.

65 Enk AH, Jonuleit H, Saloga J and Knop J: Dendritic cells as mediators of tumor-induced tolerance in metastatic melanoma. Int J Cancer *73*: 309-316, 1997.

66 Steinbrink K, Wolfl M, Jonuleit H, Knop J and Enk AH: Induction of tolerance by IL-10-treated dendritic cells. J Immunol *159*: 4772-4780, 1997.

67 Ludewig B, Graf D, Gelderblom H.B, Becker Y, Kroczek RA and Pauli G: Spontaneous apoptosis of dendritic cells is effectively inhibited by TRAP (CD40-ligand) and TNF-alpha, but strongly enhanced by interleukin-10. Eur J Immunol *25*: 1943-1950, 1995.

68 Bar-Eli M: Role of interleukin-8 in tumor growth and metastasis of human melanoma. Pathobiology *67*: 12-18, 1999.

69 Menetrier-Caux C, Montmain G, Dieu MC, Bain C, Favrot MC, Caux C and Blay JY: Inhibition of the differentiation of dendritic cells from $CD34^+$ progenitors by tumor cells: role of interleukin-6 and macrophage colony-stimulating factor. Blood *92*: 4778-4791, 1998.

70 Ebrahimi B, Tucker SL, Li D, Abbruzzese JL and Kurzrock R: Cytokines in pancreatic carcinoma, correlation with phenotypic characteristics and prognosis. Cancer *101*: 2727-2736, 2004.

71 Lauta VM: A review of the cytokine network in multiple myeloma, diagnostic, prognostic, and therapeutic implications. Cancer *97*: 2440-2452, 2003.

72 Herbewal JR, Lelievre E, Lambert C, Dy M and Genin C: Recruitment of STAT3 for production of IL-10 by colon carcinoma cells induced by macrophage-derived IL-6. J Immunol *172*: 4630-4636, 2004.

73 Balkwill F: Cancer and the chemokine network. Nature Rev Cancer *4*: 540-550, 2004.

74 Bareus PJ: Cytokine-directed therapies for the treatment of chronic airway diseases. Cytokines Growth Factor Rev *14*: 511-522, 2005.

75 Van de loosdrecht AA Huijgens PC and Ossenkoppele S: Emerging antibody-targeted therapy in leukemia and lymphoma: current concepts and clinical implicatioins. Anticancer Drugs *15*: 189-201, 2004.

76 Mocellin S, Rossi CR, Pilati P and Nitti D: Tumor necrosis factor, cancer and anticancer therapy. Cytokines Growth Factor Rev *16*: 35-53, 2005.

77 Tsimberidou AM, Waddelos T, Kautarjian HM, Albitar M and Giles FJ: Pilot study of recombinant human soluble tumor necrosis factor (TNF) receptor (p75) fusion protein (TNFR-Fc; Enbrel) in patients with refractory multiple myeloma: increase in plasma TNF alpha levels during treatment. Leuk Res *27*: 375-380, 2003.

78 Alms WJ, Atarmas SP, Yurovsky VV and White B: Generation of a variant of human interleukin-4 by alternative splicing. Mol Immunol *33*: 361-370, 1996.

79 Klein SC, Golverdingen JG, van Wichen DF, Bouwens AGM, Stuij I, Tilanus MGJ, Bast EJEG and de Weger RA: Expression of two interleukin 4 mRNA isoforms in B lymphoid cells. Cell Immunol *167*: 259-268, 1996.

80 Atamas SP, Choi J, Yurovsky VV and White B: An alternative splice variant of human IL-4, IL-4delta2, inhibits IL-4-stimulated T cell proliferation. J Immunol *156*: 435-441, 1996.

81 Arinobu Y, Atamas SP, Otsuka T, Niizo H, Yamaoka K, Mitsuyasu H, Niho Y, Hamasaki N, White B and Izuhara K: Antagonistic effects of an alternative splice variant of human IL-4, IL-2delta2 on IL-4 activities in human monocytes and B cells. Cellular Immunol *191*: 161-167, 1999.

82 Demissie A, Abebe M, Aseffa A, Rook G, Fletcher H, Zumla A, Weldingh K, Brock I, Andersen P, Mark-Doherty T and the VACSEL study group: Healthy individuals that control latent infection with *Mycobacterium tuberculosis* express high levels of Th1 cytokines and IL-4 antagonist IL-4delta2. J Immunol *172*: 6938-6943, 2004.

83 Alberti L, Bachelot T, Duc A, Biota C and Blay JY: A spliced isoform of interleukin 6 mRNA produced by renal cell carcinoma encodes for an interleukin 6 inhibitor. Cancer Res *65*: 2-5, 2005.

84 Yatsenko OP, Filipenko ML, Khrapov EA, Voronina EN, Kozlov VA and Sennikov SV: Alternative splicing of mRNA of mouse interleukin-4 and interleukin-6. Cytokine *28*: 190-196, 2004.

85 Yang Z, Feng J, Hu M, Li Y, Qin W and Shen B: A novel hIL-6 antagonist peptide from computer-aided design contributes to suppression of apoptosis in M1 cells. Biochem Biophys Res Comm *325*: 518-524, 2004.

86 Nishimoto N and Kishimoto T: Inhibition of IL-6 for treatment of inflammatory diseases. Curr Opin Pharmacol *4*: 386-391, 2004.

87 Brakenhoff JPJ, de Hon FD, Fontaine V, ten Boekel E, Schooltink H, Rose-Jones S, Heinrich PC, Content J and Aarden LA: Development of a human interleukin-6 receptor antagonist. J Biol Chem *269*: 86-93, 1994.

88 Renné C, Kallen K-J, Müllberg J, Jostock T, Grötzinger J and Rose-Jones S: A new type of cytokine receptor antagonist. J Biol Chem *273*: 27213-27219, 1998.

89 McCarthy BA, Mansour A, Lin Y-C, Kotenko S and Raveche E: RNA interference of IL-10 in leukemic B cells. Cancer Immunity *4*: 6-22, 2004.

90 Brassad DL, Grace MJ and Bordens RW: Interferon-alpha as an immunotherapeutic protein. J Leukoc Biol *71*: 565-581, 2002.

91 Becker Y: A point of view: HIV-1/AIDS is an allergy but CpG ODN treatments may inhibit virus replication and reactivate the adaptive immunity. Hypothesis and implicationis. Virus Genes *30*: 1, 127-131, 2005.

92 Vicari AP, Chiodoni C, Vaure C, Ait-Yahia S, Dercamp C, Matsos F, Reynard O, Taverne C, Merle P, Colombo MP, O'Garra A, Trichieri G and Caux C: Reversal of tumor-induced dendritic cell paralysis by CpG immunostimulatory oligonucleotide and anti-interleukin 10 receptor antibody. J Exp Med *196*: 541-549, 2002.

93 Hartmann E, Wollenberg B, Rothenfusser, S, Wagner M, Wellisch D, Mack B, Giese T, Gires O, Enders F and Hartmann G: Identification and functional analysis of tumor-infiltrating plasmacytoid dendritic cells in head and neck cancer. Caner Res *63*: 6478-6487, 2003.

94 Kemp TJ, Elzey BD and Griffith TS: Plasmacytoid dendritic cell-derived IFN-alpha induces TNF-related inducing ligand/Apo-2L-mediated antitumor activity by human monocytes following CpG oligodeoxynucleotide stimulation. J Immunol *171*: 212-218, 2003.

95 Nakamori M, Iwahashi M, Nakamura M, Ueda K, Zhang X and Yamaue H: Intensification of antitumor effect by T helper 1-dominant adoptive immunogene therapy for advanced orthopedic colon cancer. Clin Cancer Res *9*: 2357-2365, 2003.

96 Kawarada Y, Ganss R, Garbi N, Sacher T, Arnold B and Hammerling GJ: NK- and CD8+ T cell-mediated eradication of established tumors by peritumoral injection of CpG-containing oligodesoxynucleotides. J Immunol *167*: 5247-5253, 2001.

97 Heckelsmiller K, Rall K, Beck S, Schlamp A, Seiderer J, Jahlsdorfer B, Krug A, Rothenfusser S, Enders S and Hartmann G: Peritumoral CpG DNA elicits a coordinated response of CD8 T cells and innate effectors to cure established tumors in a murine colon carcinoma model. J Immunol *169*: 3892-3899, 2002.

98 Lonsdorf AS, Kuekrek H, Stern BV, Boehm BO, Lehmann PV and Tary-Lehmann M: Intratumor CpG-oligodeoxynucleotide injection induces protective antitumor T cell immunity. J Immunol *171*: 3941-3946, 2003.

99 Hafner M, Zawarzky R, Hirtreiter C, Buurman WA, Echtenacher B, Hehlgans T and Manuel DN: Antimetastatic effect of CpG DNA-mediated type I IFN. Cancer Res *61*: 5523-5528, 2001.

100 Carpentier AF, Chen L, Maltoni F and Delattre J-Y: Oligodeoxynucleotides containing CpG motifs can induce rejection of a neuroblastoma in mice. Cancer Res *59*: 5429-5432, 1999.

101 Carpentier AF, Xie J, Mokharti K and Delattre J-Y: Successful treatment of intracranial gliomas in rat by oligodeoxynucleotides containing CpG motifs. Clin Cancer Res *6*: 2469-2473, 2000.

102 Brunner C, Seiderer J, Schlamp A, Bidlingmaier M, Eigler A, Haimerl W, Lehr H-A, Krieg AM, Hartmann G and Enders S: Enhanced dendritic cell maturation of TNF-alpha or cytidine-phosphate-guanosine DNA drives T cell activation *in vitro* and therapeutic anti-tumor immune responses *in vivo*. J Immunol *165*: 6278-6286, 2000.

103 Ballas ZK, Krieg AM, Warren T, Rasmussen W, Davis HL, Waldschmidt M and Weiner GJ: Divergent therapeutic and immunologic effects of oligodeoxynucleotides with distinct CpG motifs. J Immunol *167*: 4878-4886, 2001.

104 Wooldridge JE, Ballas Z, Krieg AM and Weiner GJ: Immunostimulatory oligodeoxynucleotides containing CpG motifs enhance the efficacy of monoclonal antibody therapy of lymphoma. Blood 89: 2994-2998, 1997.

105 van Ojik HH, Bevaart L, Dahle CE, Bakker A, Jansen MJH, van Vugt MJ, van de Winkel JGJ and Weiner GJ: CpG-A and B olideoxynucleotides enhance the efficacy of antibody therapy by activating different effector cell populations. Cancer Res *63*: 5595-5600, 2003.

106 Heckelsmiller K, Beck S, Rall K, Sipos B, Schlamp A, Tuma E, Rothenfusser S, Enders S and Hartmann G: Combined dendritic cell- and CpG oligonucleotide-based immune therapy cures large murine tumors that resist chemotherapy. Eur J Immunol *32*: 3235-3245, 2002.

107 Perdigon G, de Moreno de LeBlanc A, Valdez J and Rachid M: Role of yogurt in the prevention of colon cancer. Eur J Clin Nutr *56, Supp. 3*: S65-S68, 2002.

108 de Moreno de LeBlanc A and Perdigon G: Yogurt feeding inhibits promotion and progression of experimental colorectal cancer. Med Sci Monit *10*: BR96-104, 2004.

109 Sudo N, Aiba Y, Oyama N, Yu X-N, Matsunaga M, Koga Y and Kubo C: Dietary nucleic acid and intestinal microbes synergistically promote a shift in the Th1/Th2 balance toward Th1 skewed immunity. Int Arch Allergy Immunol *135*: 132-135, 2004.

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