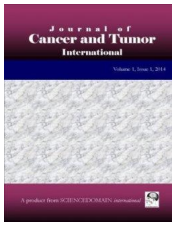

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Combined Intralipid-urotherapy for Patients with Cancer: A Creative Review

Joseph Eldor[1*]

[1]*Theoretical Medicine Institute, Jerusalem, Israel.*

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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Review Article

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ABSTRACT

The article relates in general to intralipid and its use in cancer therapy. Specifically, it relates to the combined intralipid-urotherapy for treating cancer, and methods of such treatment.
Cancer cells release various antigens, some of which appear in the urine. Combined oral Intralipid and auto-urotherapy is suggested as a new treatment modality for cancer patients. It will provide the intestinal lymphatic system the many tumor antigens against which antibodies may be produced. These antibodies may be transpierced through the blood stream and attack the tumor and its cells.
Intralipid can increase the response to the cancer antigens in the intestinal lymphatic system against which antibodies may be produced.

Keywords: Cancer; auto- urotherapy; intralipid.

1. THE PHILOSOPHY OF CANCER

Microbes were known long before the germ theory of disease was invented. It was not the discovery of germs that revolutinized medicine, but the invention of a philosophy of medical explanation that permitted germs to be causative agents of disease [1].

**Corresponding author: Email: csen_international@csen.com;*

Burnet and Thomas [2] postulated that specific cell mediated immunity may have evolved in vertebrates specially for defense against the "enemy within" rather than against infecting microorganisms and parasites. Most human cancers appear to lack truly tumor-specific antigens. The same neoplastic cell can express several different tumor antigens. For example, relatively cross-reacting tumor-specific transplantation antigens have been demonstrated in many chemically induced tumors [3].

Tumor-associated differentiation antigens are shared by neoplastic and embryonic cells [4]. The extent to which human patients react immunologically against their cancers has been a subject of much controversy [5]. Paul Ehrlich, in 1909, said: *"I am convinced that during development and growth malignant cells arise extensively frequently but that in the majority of people they remain latent due to the protective action of the host. I am also convinced that this natural immunity is not due to the presence of antimicrobial bodies but is determined purely by cellular factors. These may be weakened in the older age groups in which cancer is more prevalent"* [6].

2. TUMOR ANTIGENS IN URINE

Human melanoma cells express membrane antigens distinct from those of the normal ectodermal counterparts [7]. Urinary-tumor-associated antigen (U-TAA) is one such antigen. This high-molecular weight glycoprotein was first described when melanoma urine was found to react with autologous antibody [8]. The antigen has since been detected in the urine of 68% of melanoma patients. In addition, high levels of U-TAA are found to correlate positively with disease occurrence in surgically treated patients [9].

Prostatic specific antigen (PSA) has become an important laboratory test in the management of prostate cancer. PSA levels can be as readily obtained from voided urine as from serum samples [10].

Quantitative urinary immunocytology with monoclonal antibody (mab) 486p 3/12 proved to be valuable for diagnostic use in bladder-cancer patients` urine, especially in the followup of patients with superficial bladder carcinoma [11].

Quantitative urinary immunocytology is a general tool to test the diagnostic usefulness of monoclonal antibody (mabs), assuming that normal and malignant cells differ in their quantitative expression of a given antigen. Selective criteria for selecting mabs for diagnostic approaches should ask not for tumor specificity, but for different quantitative expression of antigen in the tissues or cells in question.

Gastric juice oncofetal antigen determination, due to direct shedding of antigens into the fluid around tumor tissues, appears to accurately indicate the presence and degree of gastric mucosal damage and to be to a slight extent influenced by unrelated factors [12]. Patients` age, for example, modifies CEA serum levels [13]. A monoclonal antibody (mab) against a human colorectal adenocarcinoma cell line has been raised [14], which reacts with sialosylfucosyllactoteraose [15] corresponding to the sialylated blood group antigen Lewis (a). The antigen defined by this antibody, CA50, is elevated in the serum of many patients with gastrointestinal tumors [16], with a sensitivity for gastric cancer ranging from 20% [17] to 65% [18]. CA50 (a tumor-associated gangliosidic antigen) levels have been determined by an RIA test in serum, gastric juice and urine of patients undergoing upper gastrointestinal tract endoscopy. Sensitivity and specificity were respectively 23% and 89% for CA50 determination in urines [19].

Soluble forms of membrane proteins such as cytokine receptors or cellular adhesion molecules (CD14, TNF receptor, CD25, IL-6 receptor, IFN-γ-receptor and CD54) have been detected in human body fluids. They may have important functions in immune regulation by blocking receptor/ligand interactions. The human adhesion receptor CD58 (LFA-3) is expressed on most cell types. A soluble form of CD58 (sCD58) was purified from human urine and partially purified from supernatant of the Hodgkin-derived cell line L428 [20].

Urinary organ-specific neoantigen from colorectal cancer patients has been used to make a monoclonal antibody, BAC 18.1 [21]. Organ-specific neoantigen originates in the colon and is excreted into the urine, so the BAC 18.1 binding levels in the urine may be a diagnostic aid for colorectal cancer.

The polyamines spermidine, spermine and their diamine precursor putrescine are ubiquitous constituents of mammalian cells that are fundamentally involved in normal, malignant and induced proliferative states. The polyamines and ornithine decarboxylase (ODC), the rate-limiting enzyme of the polyamine metabolism, were found to play an important role in tumor promotion [22]. The suggestion that polyamines play an important role in colorectal cancer was confirmed by studies that found elevated polyamine concentrations in blood or urine [23] of patients with colon carcinoma. Sensitivity of urinary polyamines for colon cancer were highest for total spermidine (92.1%), acetylated putrescine (84.5%), total putrescine (84.0%), N1-acetylspermidine (79.3%) and N8-acetylspermidine (78.6%), but in all these cases specificity was lower than 65% (24). In patients with successful curative surgical treatment all preoperatively elevated urinary polyamine concentrations markedly decreased and returned to normal, whereas they were elevated and increased further in patients with proven relapse of the tumor and/or metastases in different organs [24].

The function of the CD44 gene is severely damaged, beginning with the very early pre-invasive stages of tumor development. This can be used as a means of tumor detection and diagnosis both on solid tissue specimens [25] and on exfoliated cells in clinically obtained excreta and body fluids [26]. Urine cell lysates obtained from patients with bladder cancer can be discriminated from normal urine lysates [27] using Western blotting with a monoclonal antibody against the standard form of the CD44 protein.

3. IMMUNOTHERAPY

Zbar and Tanaka [28] first reported on animal immunotherapy based on the principle that tumor growth is inhibited at sites of delayed hypersensitivity reactions provoked by antigens unrelated to the tumor. They injected living Mycobacterium bovis (strain BCG) into established intradermal tumors and caused tumor regression and prevented the development of metastases. For optimum therapeutic effect contact between BCG and tumor cells was necessary.

The ability of tumor immune lymphocytes to localize specifically to tumor offers a possibility for therapy which has been utilized over the past several years [29]. The rejection of murine tumors expressing tumor-specific transplantation antigens has been shown to be mediated primarily by immune cells [30]. Some 6 to 7% of transplant recipients may develop cancer as a consequence of iatrogenic immunosuppression [31]. Studies on the ability of patient lymphocytes to lyse tumor cells in short term (2-8 hr) isotope release assays have shown that lymphocytes from cancer patients can generally destroy only tumor cells from the same patient [32-34], unless the effector cells are not cytolytic T cells but, for example, Natural Killer cells or Lymphokine Activated Killer cells, in which case neoplastic cells representing many different types are sensitive.

Immunotherapy is believed to be capable of eliminating only relatively small amounts of neoplastic cells and, therefore, the failure to induce a regression in patients with excessive tumor burden is not unexpected [35,36]. One approach of immunotherapy is to "xenogenize" tumor cells by virus infection. Another is to culture tumor infiltrating lymphocytes with interleukin-2 and reinoculate them into the host with cytokines [37]. The introduction of recombinant vectors expressing cytokine genes into tumor infiltrating lymphocyte cells [38] or into the tumor cells themselves [39] may enhance the migration of effector immune cells into the tumor with consequent immunomediated control. The considerable heterogeneity in the expression of tumor associated differentiation antigens by cells within the same tumor constitutes a problem for any immunotherapy, since it facilitates the escape of antigen-negative tumor variants.

An alternative approach toward increasing the immune response to tumor-associated differentiation antigens is to treat the host to be immunized so as to abolish a "suppressor" response. Such treatment can be provided in the form of sublethal whole body x-irradiation [40], injection of a drug such as cyclophosphamide [41], or by the administration of certain anti-idiotypic antibodies [42].

Anergy is defined as a state of T lymphocyte unresponsiveness characterized by absence of proliferation,IL-2 production and diminished expression of IL-2R [43,44]. Most available data support suppression as a mechanism of oral tolerance [45,46]. Immunological suppression is classically demonstrated by the suppression of

antigen-specific immune responses by T lymphocytes [47,48].

4. AUTOANTIGENS

Oral administration of S-antigen (S-Ag), a retinal autoantigen that induces experimental autoimmune uveitis, prevented or markedly diminished the clinical appearance of S-Ag-induced disease as measured by ocular inflammation [49,50].

Gut associated lymphoid tissue has the capacity to generate potent immune responses on one hand, and to induce peripheral tolerance to external antigens on the other [51-53]. Both processes require antigen stimulation [53], involve cytokine production [51] and might occur at the same time - the first leading to potent local and systemic immune responses, while the latter leads to systemic antigen-specific nonresponsiveness [54]. The generation of acquired immune responses in the small intestine is believed to occur in Peyer`s patches [51,55].

Orally fed protein antigens are found in the blood within 1 hr of feeding [56]. Peripheral tolerance is not induced locally, but rather is induced systemically upon transfer of intact antigen, or its peptides, into the circulation [57-59]. Oral tolerance may be induced by a single feeding of a protein antigen [60,61] or by several intermittent feedings [46,62]. In order to test whether feeding on autoantigen could suppress an experimental autoimmune disease, the Lewis rat model of experimental autoimmune encephalomyelitis was studied [63]. With increasing dosages of GP-MBP (guinea pigs - myelin basic protein), the incidence and severity of disease was suppressed, as well as proliferative responses of lymph node cells to MBP. Antibody responses to MBP were decreased but not as dramatically as proliferative responses. Thus it appears that oral tolerance to MBP, as to other non-self antigens [45], preferentially suppress cellular immune responses. It appears that homologous MBP is a more potent oral tolerogen for experimental autoimmune encephalomyelitis than heterologous MBP [64].

Tumor cells may escape immune recognition in immunocompetent hosts by clonal evolution. Attention could be directed to activate the resident immune effectors to break the anergy or tolerance.

5. UROTHERAPY

Subcutaneous urine injections was practiced in 1912 by Duncan [65] from New York under the name of auto-pyotherapy for urinary infections, and in 1919 by Wildbolz [65] from Bern for diagnostic purposes. Cimino [66] from Palermo reported in 1927 on the use of auto uro-therapy for urinary infections. Rabinowitch [67] in 1931 described this auto-urine therapy for gonarthritis. Jausion *et al*. [68] used this kind of therapy in 1933 for desensitization and endocrinological problems. They treated with auto urotherapy injections patients who suffered from migraine, pruritus, asthma, urticaria, eczema, psoriasis, etc. Day [69] in 1936 treated patients with acute and subacute glomerulonephritis by injection of an autogenous urinary extract. Sandweiss, Saltzstein and Farbman [70] reported in 1938 that an extract from urine of pregnant women has a prophylactic and therapeutic effect on experimental ulcers in dogs. Shortly thereafter the same group noted that an extract from urine of normal women has a similar beneficial effect [71].

In 1926 Seiffert first described the construction of ileal loop conduits for urinary diversion [72]. Bricker in the 1950s popularized the use of the ileal loop as a means of supravesical urinary diversion following exenteration for pelvic malignancy in adults [73]. Ureterosigmoidostomy as a means of urinary diversion was used widely from 1920 to 1955. It was this type of implant which Hammer first reported in 1929 associated with tumor [74].

Peyer`s patches are immunocompetent lymphoid organs which participate in intestinal immune responses [75]. Epithelial cells within the crypts of the small bowel are one of the fastest dividing cells in the body and yet they show one of the lowest rate of malignant transformation [76]. Stem cells in the mucosa of the small bowel can divide every 8 to 12 hours [77]. Tapper and Folkman [78] demonstrated that exposure of intestinal segments to urine causes marked lymphoid depletion in the segments. These studies give additional support to the idea that a lymphocyte suppressive factor exist in urine [79]. The continued presence of urine bathing the intestinal mucosa appears to locally inhibit regeneration of the Peyer`s patches.

Starkey et al. [80] detected in human urine a material that is biologically and immunologically

similar to epidermal growth factor that causes proliferation and keratinization of epidermal tissues.

The increased susceptibility of the colon to cancer associated with the existence of an implanted ureter has been theorized to relate to 3 factors: 1. The role of the urine in the colon [81,82]; 2. The mechanical effect of the fecal stream on the stoma [83]; and 3. The age of the anastomosis [84]. Adenocarcinoma of the colon mucosa is a recognized complication of ureterosigmoidostomy. The tumor, which develops adjacent to the junction of the ureter with the bowel, occurs 500 times as often as in the population at large and, in children so operated , 7,000 times as often as in all persons under age 25. The latency period is 5 to 50 years [81,85-87].

It is common knowledge that malignant tumors may disappear spontaneously although very infrequently [88-90]. Usually it is accepted that this could be due at least partly to an immunological reaction [91,92]. Renal adenocarcinoma is one of the cancer types in which such spontaneous regressions have been described most frequently [88,90].

Urinary extracts from patients with aplastic anemia [93] and idiopathic thrombocytopenic purpura [94] are capable of stimulating megakaryocyte colony growth in culture, and when injected into rats could also induce thrombocytosis in peripheral blood and megakaryocytosis in the spleens of these animals. Stanley et al. [95] demonstrated that rabbits immunized with human urine concentrate from leukemia patients developed antibody which neutralized the mouse bone marrow colony stimulating factor in human urine and human serum.

Malignant tumors express antigens that may stimulate and serve as targets for antitumor immunity. Virally induced tumors usually contain integrated proviral genomes in their cellular genomes and often express viral genome-encoded proteins that may stimulate specific host immune responses. Antigens unique to individual tumors that stimulate specific rejection of transplanted tumors have been demonstrated only in experimental animals. Other tumor antigens that potentially can stimulate immune responses are shared by different tumors. These include products of mutated or rearranged oncogenes or tumor-suppressor genes. Tumors may also overexpress tissue differentiation antigens or embryonic antigens, which also have the potential to be recognized by the immune system. The recent identification of tumor antigens recognized by cytotoxic T cells opens up new possibilities for constructing chemically defined antigens for specific immunotherapy. Treatment of malignant tumors in humans by immunologic approaches, although theoretically attractive, has not yet succeeded on a large scale. Important progress in immunotherapy of cancer is emerging with several different treatment modalities [97].

Recent studies have identified new melanoma antigens that are recognised by CD4(+) T cells. Analysis of tumour-specific CD4(+) T-cell responses may lead to the development of optimal anti-cancer vaccines that can induce an orchestrated effort of tumour-specific CD4(+) and CD8(+) T cells in the fight against cancer [98].

T cells play an important role in in vivo rejection of human melanoma. Human melanoma antigens recognized by autologous T cells were identified. These antigens are classified as tissue (melanocyte)-specific proteins, cancer-testis antigens (proteins expressed in normal testis and various cancers), tumor-specific peptides derived from mutations in tumor cells, and others. A variety of mechanisms generating T cell epitopes on tumor cells were discovered. Various clinical observations, including tumor regression observed in adoptive transfer of gp100-reactive T cells suggest that these identified melanoma peptides may function as tumor rejection antigens. Immunodominant common epitopes that could expand melanoma-reactive cytotoxic T lymphocytes (CTLs) in vitro were found in the MART-1 and gp100 antigens. New immunization protocols--including immunization with peptides, recombinant viruses, plasmid DNAs, and dendritic cells pulsed with peptides as well as adoptive transfer of in vitro-generated CTLs by stimulation with antigenic peptides--were developed (phase I clinical trials have been performed in the Surgery Branch of the National Cancer Institute, Bethesda, MD, U.S.A.). Immunization with the gp100(209(210M)) peptide that was modified to have high HLA-A2 binding affinity, along with incomplete Freund's adjuvant and interleukin (IL)-2, resulted in a 42% response rate in patients with melanoma. These immunotherapies need further improvement due to the mechanisms of tumor escape from T cell responses [99].

Most major advances in human cancer immunology and immunotherapy have come from studies in melanoma. We are beginning to understand the immune repertoire of T cells and antibodies that are active against melanoma, with recent glimpses of the CD4(+) T cell repertoire. The view of what the immune system can see is extending to mutations and parts of the genome that are normally invisible [100].

Pancreatic cancer is the fifth leading cause of cancer deaths in the United States with little or no impact from conventional treatment options. Significant advances in understanding basic immunology have renewed interest in using immunotherapy to treat pancreatic cancer. Cancer immunotherapy, including humanized MAbs, cytokines, and potent vaccine strategies, has been successful in animal models and is being evaluated in clinical trials. Gene therapy is also being explored using methods to inactivate oncogenes, replace defective tumor suppressor genes, confer enhanced chemosensitivity to tumor cells, and increase immunogenicity of tumor cells. Angiogenesis, an essential step in the growth and metastasis of pancreatic cancer, has been targeted by many antiangiogenic agents. Several clinical trials have been initiated to evaluate the role of these innovative strategies in patients with pancreatic cancer with increasingly sophisticated correlative studies to learn more about the mechanisms of tumor rejection with these agents. The rapid translation of basic science discoveries to clinical trials should result in the development of new effective treatments for patients with pancreatic cancer [101].

The immune repertoire contains T cells and B cells that can recognize autologous cancer cells. This repertoire is directed against self, and in some cases altered self (mutations). Priming immune responses against self antigens can be difficult. Strategies are presented using altered self to elicit immunity against self in poorly immunogenic tumor models. Mechanisms underlying immunity to self antigens on cancer cells show that the immune system can use diverse strategies for cancer immunity, in both the immunization and the effector phases. CD4+ T cells are typically, but not always, required for immunization. The effector phase of tumor immunity can involve cytotoxic T cells, macrophages with activating Fc receptors, and/or killer domain molecules. This diversity in the effector phase is observed even when immunizing with conserved paralogs. A consequence of tumor immunity is potentially autoimmunity, which may be undesirable. Autoimmunity uses similar mechanisms as tumor immunity, but tumor immunity and autoimmunity can uncouple. These studies open up strategies for active immunization against cancer [102].

6. CANCER ANTIGENS

The spectrum of human antigens allows a monitoring of various pathological processes such as autoimmune disorders and tumorigenesis. Serological analysis of cDNA expression libraries (SEREX) is now used to search for new cancer-associated antigens, which are potential diagnostic markers or targets for immunotherapy of cancer [103].

The immune response can effectively hamper the progression of preclinical stages of tumor growth. Medicine in the postgenomic era offers an increasing possibility of detecting healthy individuals at risk of developing cancer who could benefit from tumor-preventive vaccines. The identification of novel tumor antigens that fulfill two conditions will be crucial for the development of cancer immunoprevention. First, an ideal antigen should have a crucial pathogenetic role in tumor growth to avoid the selection of antigen-loss variants. Second, the antigen should be recognizable by the immune system even in MHC-loss variants and should therefore be recognized both by antibodies and T cells. Identifying such antigens will also provide new targets for cancer immunotherapy [104].

Cancer/testis (CT) antigens are a category of tumor antigens with normal expression restricted to male germ cells in the testis but not in adult somatic tissues. In some cases, CT antigens are also expressed in ovary and in trophoblast. In malignancy, this gene regulation is disrupted, resulting in CT antigen expression in a proportion of tumors of various types. Since their initial identification by T-cell epitope cloning, the list of CT antigens has been greatly expanded through serological expression cloning (SEREX) and differential mRNA expression analysis, and approximately 20 CT antigens or antigen families have been identified to date. Characteristics commonly shared by CT antigens, aside from the highly tissue-restricted expression profile, include existence as multigene families, frequent mapping to chromosome X, heterogeneous protein expression in cancer, likely correlation with tumor progression, induction of expression

by hypomethylation and/or histone acetylation, and immunogenicity in cancer patients. Spontaneous humoral and cell-mediated immune responses have been demonstrated against several CT antigens, including NY-ESO-1, MAGE-A, and SSX antigens. Since CT antigens are immunogenic and highly restricted to tumors, their discovery has led directly to the development of antigen-specific cancer vaccines, and clinical trials with MAGE-A and NY-ESO-1 are in progress [105].

Our understanding of how immune responses are generated and regulated drives the design of possible immunotherapies for cancer patients. Cancer vaccines that are able to induce tumor-specific immune responses in cancer patients are not always followed by tumor rejection. Two possible reasons that might explain this dichotomy of cancer immunology. First, the immune response generated, although detectable, may not be quantitatively sufficient to reject the tumor. Second, the tumor microenvironment may modulate tumor cell susceptibility to the systemic immune response induced by the immunization [106].

Cytolytic T lymphocytes (CTL) play a major role in the recognition and destruction of tumor cells by the immune system. Some of these antigens, including those encoded by the MAGE genes, are absent on all normal cells, and therefore constitute ideal targets for cancer vaccines aimed at increasing the activity of anti-tumor lymphocytes. Such vaccines are currently tested in clinical trials with melanoma patients. These antigens consist of small peptides that are presented by HLA molecules and that result from the degradation of intracellular proteins. This degradation is performed by an intracellular proteolytic complex called the proteasome. Dendritic cells, which in the lymph node are responsible for antigen presentation to the lymphocytes in order to initiate the immune response, are inefficient to produce some peptides because they contain a different proteasome called "immunoproteasome" [107].

One of the most significant advances in the field of modern tumor immunology is the identification of genes encoding tumor-rejection antigens that are recognized by human leukocyte antigen (HLA) class I-restricted and tumor-specific cytotoxic T lymphocytes (CTLs). Several peptides encoded by these genes are now under clinical trial as cancer vaccines, and major tumor regression has been observed in some melanoma patients. These results indicate that identification of the peptides capable of inducing CTLs may provide a new modality of cancer therapy. Itoh et al. [108] investigated tumor-rejection antigens from epithelial cancers, and reported 7 genes encoding tumor-rejection antigens and peptides available for specific immunotherapy of HLA-A26 or -A24 patients with epithelial cancers. Furthermore, they identified more than 10 genes encoding tumor-rejection antigens and peptides available for specific immunotherapy of HLA-A2 patients with epithelial cancers. Therefore these new antigens and peptides could be applicable to the treatment of numerous epithelial cancer patients.

Cytotoxic T-cell responses to shared tumor antigens have been characterized for several tumor types, and the MHC-associated peptides that comprise these antigens have been defined at a molecular level. These provide new tools to determine whether immune responses can be generated with these tumor antigens, and there are data to suggest that such immune responses can be generated. However, it is also clear that tumor cells can evade immune responses directed against some shared antigens, by downregulating expression of MHC or of the antigenic protein(s), as well as by more active methods such as secretion of immunosuppressive cytokines. Awareness of these mechanisms of immune escape will help to direct development of the next generation of tumor vaccines. Targeting unique antigens and modulating the cytokine environment likely will be critical to comprehensive vaccine systems in the future [109].

The adoptive transfer of tumor-infiltrating lymphocytes along with interleukin 2 into autologous patients resulted in the objective regression of tumor in about 30% of patients with melanoma, indicating that these T cells play a role in tumor rejection. To understand the molecular basis of the T cell-cancer cell interaction Wang [110] and others started to search for tumor antigens expressed on cancer cells recognized by T cells. This led to the identification of several major histocompatibility complex (MHC) class I restricted tumor antigens. These tumor antigens have been classified into several categories: tissue-specific differentiation antigens, tumor-specific shared antigens, and tumor-specific unique antigens. Because CD4+ T cells play a central role in orchestrating the host immune response against cancer, infectious diseases, and autoimmune diseases, a novel

genetic approach has recently been developed to identify these MHC class II restricted tumor antigens. The identification of both MHC class I and II restricted tumor antigens provides new opportunities for the development of therapeutic strategies against cancer.

In order to enhance cell mediated cytotoxicity, bispecific antibodies (BsAbs), molecules combining two or more antibodies with different antigenic specificities, have been developed as new agents for immunotherapy. Kudo et al. [111] recent studies revealed that simultaneous administration of two kinds of BsAbs (anti-tumor x anti-CD3 plus anti-tumor x anti-CD28) together with lymphokine activated killer cells with a T cell phenotype (T-LAK cells) inhibited growth of human xenotransplanted tumors in severe combined immunodeficient (SCID) mice, while single BsAb was without effect. Three kinds of BsAbs (anti-tumor x anti-CD3, anti-tumor x anti-CD28, anti-tumor x anti-CD2) showed the highest cytotoxicity against tumor cells when given simultaneously with T-LAK cells or peripheral blood mononuclear cells in vitro and in vivo. BsAbs can be preserved for immediate application, while cytotoxic T lymphocytes (CTLs) must be made-to-order, and are time-consuming to prepare. Tumor associated antigens, such as MAGE antigens, SART antigens, MUC1 antigen, c-erbB 2 antigen or cancer/testis antigens can be served to target antigens for BsAb production. By conjugation with antibodies to effector cells (anti-CD3, anti-CD28, anti-CD16, anti-CD64, anti-CD89 or anti-CD2), many kinds of BsAbs can be produced to cover most types of cancers from different organs. Therefore this strategy might be ubiquitously applicable to most malignancies.

Melanogenesis-related proteins play important roles in melanin synthesis and antigenicity of melanomas. Identification of highly expressed melanoma-associated antigens (MAA) that are immunogenic in humans will provide potential targets for cancer vaccines. Melanogenesis-related proteins have been shown to be MAA. Autoantibody responses to these MAA have been shown to react with melanoma cells and melanocytes, and suggested to play a role in controlling melanoma progression. To assess antibody responses to potential melanoma/melanocyte autoantigens, the open-reading frame sequences of tyrosinase, tyrosinase-related protein (TRP)-1, TRP-2, and melanoma-associated glycoprotein antigen family (gp100/pmel17) genes were cloned and expressed as recombinant proteins in *E. coli* [112]. Purified recombinant antigens were employed to detect antibodies in sera of melanoma patients and normal healthy donors. By affinity enzyme-linked immunosorbent assay and western blotting, all recombinant antigens were shown to be antigenic. The main subclass of antibody response to these antigens was IgG. Most importantly this study demonstrated anti-TRP-2 and anti-gp100/pmel17 IgG responses in melanoma patients. Only one of 23 normal donors had an antibody response to the antigens tested. MAA-specific IgG antibodies in sera were assessed in melanoma patients (n = 23) pre- and post-polyvalent melanoma cell vaccine treatment. Polyvalent melanoma cell vaccine treatment enhanced anti-MAA antibody responses; however, only anti-TRP-2 and anti-gp100/pmel17 antibody response was enhanced. These studies suggest that four melanogenesis-related proteins are autoimmunogenic and can be used as potential targets for active-specific immunotherapy.

The adoptive transfer of cytotoxic T lymphocytes (CTLs) derived from tumor-infiltrating lymphocytes (TIL) along with interleukin 2 (IL-2) into autologous patients with cancer resulted in the objective regression of tumor, indicating that these CTLs recognized cancer rejection antigens on tumor cells. To understand the molecular basis of T cell-mediated antitumor immunity, several groups started to search for such tumor antigens in melanoma as well as in other types of cancers. A number of tumor antigens were isolated by the use of cDNA expression systems and biochemical approaches. These tumor antigens could be classified into several categories: tissue-specific differentiation antigens, tumor-specific shared antigens, and tumor-specific unique antigens. However, the majority of tumor antigens identified to date are nonmutated, self-proteins. This raises important questions regarding the mechanism of antitumor activity and autoimmune disease. The identification of human tumor rejection antigens provides new opportunities for the development of therapeutic strategies against cancer [113].

7. CANCER VACCINES

Multiple novel immunotherapy strategies have reached the stage of testing in clinical trials that were accelerated by recent advances in the characterization of tumor antigens and by a more precise knowledge of the regulation of cell-mediated immune responses. The key steps in

the generation of an immune response to cancer cells include loading of tumor antigens onto antigen-presenting cells in vitro or in vivo, presenting antigen in the appropriate immune stimulatory environment, activating cytotoxic lymphocytes, and blocking autoregulatory control mechanisms. This knowledge has opened the door to antigen-specific immunization for cancer using tumor-derived proteins or RNA, or synthetically generated peptide epitopes, RNA, or DNA. The critical step of antigen presentation has been facilitated by the coadministration of powerful immunologic adjuvants, the provision of costimulatory molecules and immune stimulatory cytokines, and the ability to culture dendritic cells. Advances in the understanding of the nature of tumor antigens and their optimal presentation, and in the regulatory mechanisms that govern the immune system, have provided multiple novel immunotherapy intervention strategies that are being tested in clinical trials [114].

The critical role of antigen-specific T cells in cancer immunotherapy has been amply demonstrated in many model systems. Though success of clinical trials still remains far behind expectation, the continuous improvement in our understanding of the biology of the immune response will provide the basis of optimized cancer vaccines and allow for new modalities of cancer treatment. The future will mainly be concerned with allogeneic bone marrow cell transplantation after non-myeloablative conditioning, because this approach could provide a major breakthrough in cancer immunotherapy [115]. Concerning active vaccination protocols the following aspects will be addressed: i) the targets of immunotherapeutic approaches; ii) the response elements needed for raising a therapeutically successful immune reaction; iii) ways to achieve an optimal confrontation of the immune system with the tumor and iv) supportive regimen of immunomodulation. Many questions remain to be answered in the field of allogeneic bone marrow transplantation after non-myeloablative conditioning to optimize the therapeutic setting for this likely very powerful tool of cancer therapy.

Active immunotherapy using dendritic cells (DCs) to deliver tumor antigens has generated considerable excitement among oncologists worldwide. Although most tumor antigens used in immunotherapeutic approaches are tumor-associated, often, little is known about the underlying biology of the target. Antigen expression is a prerequisite for tumor formation or maintenance by the use of 'obligate' tumor antigens. The prototype for this class of antigens is the p53 tumor antigen, which is mutated in > 50% of human malignancies. The direct involvement of p53 in the malignant transformation of tumors makes it an attractive target for immunotherapy. p53-Reactive antibodies have been found in patients with various types of cancer, demonstrating that the human immune system can recognize and respond to tumor-associated p53. Extensive preclinical experimentation has now validated the translation of p53-expressing DCs into a clinical setting. Clinical trials are ongoing to evaluate the safety and antitumor responses elicited by DCs transduced with adenoviral-p53 in cancer patients [116].

Tumor vaccination strategies have been increased over the past years. This increase began with the identification of tumor antigens recognized by the immune system. Better understanding of the immune system and increasing knowledge about the antigen presentation process and the role of dendritic cells have opened new therapeutic possibilities. DNA vaccines, already successfully used against viral antigens and covering a broad repertoire of epitopes, might also be of advantage in tumor immunotherapy. Design and selection of vectors are of considerable importance for the vaccination. There are three major types of DNA-based recombinant cancer vaccines: DNA from tumor antigens can be used 1) to modify dendritic cells, 2) as 'naked' DNA-vaccine or 3) to construct recombinant viral vaccines [117].

It is now clear that many human tumor antigens can be recognised by the immune system. These tumor antigens can be classified into several groups including cancer-testis, differentiation, tissue specific, over-expressed, and viral-associated antigens. In many cases, there is a known molecular basis of carcinogenesis which provides the explanation for the differentiated expression of these antigens in tumors compared with normal cells. Improved understanding of the biology of the immune response, particularly of immune recognition and activation of T-cells, allow better design of vaccines. Pre-clinical comparative studies allow evaluation of optimal vaccine strategies which can then be delivered to the clinic. Currently, a range of cancer vaccines are

being tested including those using tumor cells, proteins, peptides, viral vectors, DNA or dendritic cells. Ultimately, this research should give rise to an entirely new modality of cancer treatments [118].

The identification of antigens on tumor cells has led to significant contributions to the field of immunotherapy. One of the most active areas under investigation in cancer immunotherapy is the development of vaccines against melanoma antigens. Induction of immunity against tumor antigens can follow multiple routes using different mechanisms. Crucial to the development of active immunization and other immunotherapies is the discovery and understanding of the molecular identity of antigens and the mechanisms involved in tumor immunity, as well as escape from immunity [119].

Antigenic differences between normal and malignant cells form the basis of clinical immunotherapy protocols. Because the antigenic phenotype varies widely among different cells within the same tumor mass, immunization with a vaccine that stimulates immunity to a broad array of tumor antigens expressed by the entire population of malignant cells is likely to be more efficacious than immunization with a vaccine for a single antigen. One strategy is to prepare a vaccine by transfer of DNA from the patient's tumor into a highly immunogenic cell line. Weak tumor antigens, characteristic of malignant cells, become strongly antigenic if they are expressed by immunogenic cells. In animal models of melanoma and breast cancer, immunization with a DNA-based vaccine is sufficient to deter tumor growth and to prolong the lives of tumor-bearing mice [120].

Berd [121] has devised a novel approach to active immunotherapy based on modification of autologous cancer cells with the hapten, dinitrophenyl (DNP). The treatment program consists of multiple intradermal injections of DNP-modified autologous tumor cells mixed with BCG. Administration of DNP-vaccine to patients with metastatic melanoma induces a unique reaction - the development of inflammation in metastatic masses. Histologically, this consists of infiltration of T lymphocytes, most of which are CD8+. These T cells usually produce gamma interferon in situ. Moreover, they represent expansion of T cell clones with novel T cell receptor structures. Occasionally, administration of DNP-vaccine results in partial or complete regression of measurable metastases. The most common site of regression has been small lung metastases. Administration of DNP-vaccine to patients in the post-surgical adjuvant setting produces a more striking clinical effect. Berd et al. have treated 214 patients with clinically evident stage III melanoma who had undergone lymphadenectomy. With a median follow-up time of 4.4 years (1.8-10.4 years) the 5-year overall survival (OS) rate is 47% (one nodal site = 51%, two nodal sites = 33%). These results appear to be comparable to those obtained with high dose interferon. More recent studies suggest that this therapeutic approach is also applicable to ovarian cancer. There appear to be no insurmountable impediments to applying this approach to much larger numbers of patients or to developing it as a standard cancer treatment.

Certain anti-idiotypic antibodies that bind to the antigen-combining sites of antibodies can effectively mimic the three-dimensional structures and functions of the external antigens and can be used as surrogate antigens for active specific immunotherapy. Extensive studies in animal models have demonstrated the efficacy of these vaccines for triggering the immune system to induce specific and protective immunity against bacterial, viral and parasitic infections as well as tumors. Several monoclonal anti-idiotype antibodies that mimic distinct human tumor-associated antigens have been developed and characterized. Encouraging results have been obtained in recent clinical trials using these anti-idiotype antibodies as vaccines [122].

Immunization with anti-idiotype (Id) antibodies represents a novel new approach to active immunotherapy. Extensive studies in animal tumor models have demonstrated the efficacy of anti-Id vaccines in preventing tumor growth and curing mice with established tumor. Bhattacharya-Chatterjee *et al*. [123] have developed and characterized several murine monoclonal anti-Id antibodies (Ab2) which mimic distinct human tumor-associated antigens (TAA) and can be used as surrogate antigens for triggering active anti-tumor immunity in cancer patients.

Immunization with dendritic cells loaded with tumor antigens could represent a powerful method of inducing antitumor immunity. Studies from several laboratories have shown that immunization with dendritic cells pulsed with specific antigens prime cytotoxic T-cells and

engender tumor immunity. The majority of cancer patients who lack an identified tumor antigen and/or cannot provide sufficient tumor tissue for antigen preparation are excluded from treatment with cancer vaccines based on using either specific tumor antigens or mixtures of tumor-derived antigens in the form of peptides or proteins isolated from tumor cells. Vaccination with the mRNA content of tumor cells would extend the scope of vaccination to this group of patients as well because RNA can be amplified from very few cancer cells [124].

The adoptive transfer of tumor-infiltrating lymphocytes (TIL) along with interleukin (IL)-2 into autologous patients with cancer resulted in the objective regression of tumor, indicating that T cells play an important role in tumor regression. In the last few years, efforts have been made towards understanding the molecular basis of T-cell-mediated antitumor immunity and elucidating the molecular nature of tumor antigens recognized by T cells. Tumor antigens identified thus far could be classified into several categories: tissue-specific differentiation antigens, tumor-specific shared antigens and tumor-specific unique antigens. CD4+ T cells play a central role in orchestrating the host immune response against cancer, infectious diseases and autoimmune diseases. The identification of tumor rejection antigens provides new opportunities for the development of therapeutic strategies against cancer [125].

Human tumors express a number of protein antigens that can be recognized by T cells, thus providing potential targets for cancer immunotherapy. Dendritic cells (DCs) are rare leukocytes that are uniquely potent in their ability to present antigens to T cells, and this property has prompted their recent application to therapeutic cancer vaccines. Isolated DCs loaded with tumor antigen ex vivo and administered as a cellular vaccine have been found to induce protective and therapeutic anti-tumor immunity in experimental animals. In pilot clinical trials of DC vaccination for patients with non-Hodgkin's lymphoma and melanoma, induction of anti-tumor immune responses and tumor regressions have been observed. Additional trials of DC vaccination for a variety of human cancers are under way, and methods for targeting tumor antigens to DCs in vivo are also being explored. Exploitation of the antigen-presenting properties of DCs thus offers promise for the development of effective cancer immunotherapies [126].

Recently, cancer immunotherapy has emerged as a therapeutic option for the management of cancer patients. This is based on the fact that our immune system, once activated, is capable of developing specific immunity against neoplastic but not normal cells. Increasing evidence suggests that cell-mediated immunity, particularly T-cell-mediated immunity, is important for the control of tumor cells. Several experimental vaccine strategies have been developed to enhance cell-mediated immunity against tumors. Some of these tumor vaccines have generated promising results in murine tumor systems. In addition, several phase I/II clinical trials using these vaccine strategies have shown extremely encouraging results in patients [127].

Animal studies have shown that vaccination with genetically modified tumor cells or with dendritic cells (DC) pulsed with tumor antigens are potent strategies to elicit protective immunity in tumor-bearing animals, more potent than "conventional" strategies that have been tested in clinical settings with limited success. While both vaccination strategies are forms of cell therapy requiring complex and costly ex vivo manipulations of the patient's cells, current protocols using dendritic cells are considerably simpler and would be more widely available. Vaccination with defined tumor antigens presented by DC has obvious appeal. However, in view of the expected emergence of antigen-loss variants as well as natural immunovariation, effective vaccine formulations must contain mixtures of commonly, if not universally, expressed tumor antigens. When, or even if, such common tumor antigens will be identified cannot be, predicted, however. Thus, for the foreseeable future, vaccination with total-tumor-derived material as source of tumor antigens may be preferable to using defined tumor antigens. Vaccination with undefined tumor-derived antigens will be limited, however, by the availability of sufficient tumor tissue for antigen preparation. Because the mRNA content of single cells can be amplified, tumor mRNA, or corresponding cDNA libraries, offer an unlimited source of tumor antigens. DC transfected with tumor RNA were shown to engender potent antitumor immunity in animal studies. Thus, immunotherapy using autologous DC loaded with unfractionated tumor-derived antigens in the form of RNA emerges as a potentially powerful and broadly useful vaccination strategy for cancer patients [128].

8. CANCER IMMUNOTHERAPY

Adoptive immunotherapy--the isolation of antigen-specific cells, their ex vivo expansion and activation, and subsequent autologous administration--is a promising approach to inducing antitumor immune responses. The molecular identification of tumor antigens and the ability to monitor the persistence and transport of transferred cells has provided new insights into the mechanisms of tumor immunotherapy. Recent studies have shown the effectiveness of cell-transfer therapies for the treatment of patients with selected metastatic cancers. These studies provide a blueprint for the wider application of adoptive-cell-transfer therapy, and emphasize the requirement for in vivo persistence of the cells for therapeutic efficacy [129].

There is clear evidence that certain forms of immunotherapy can be successful against certain cancers. However, it would appear that cancerous cells of various origin are exceptionally adept at subverting the immune response. Consequently, it is probable that the most efficacious therapy will be one in which multiple responses of the immune system are activated. There is currently an embarrassment of riches with regard to multiple vaccine strategies in the clinic, although no single method seems to hold the solution [130].

Despite advances in chemotherapy and surgical techniques, patients with cancer often develop local recurrence or metastatic spread. Recent advances in molecular biology, coupled with new insights in tumor immunology, have led to the design of novel antitumor vaccines. Poxviruses are a large family of DNA viruses that provide an effective and safe vector system for vaccine development. The poxvirus strategy has been successfully documented in animal models, and has been used to express both tumor-associated antigens and immune stimulatory molecules [131].

Prostate cancer is the most common malignancy in American men. Metastatic prostate cancer is incurable, with the currently best treatment, androgen ablation, being only palliative. Therefore, there is a need to develop new, more effective therapies against this disease. Multiple immunotherapeutic strategies are being explored for the treatment of prostate cancer, with the hope that such treatment will be more effective and have fewer side effects than current treatment options. Several immunotherapy strategies have been shown to be effective against prostate tumors in animal models, and many of these strategies are beginning to be tested in clinical trials for their efficacy against human prostate cancer. It is likely that effective treatment of prostate cancer will require the use of both immunotherapeutic and traditional approaches in multimodality treatments. In addition, for immunotherapy to be effective against prostate cancer, ways to overcome immune evasion and immunosuppression by the tumor cells will need to be developed [132].

Despite the identification of tumor antigens and their subsequent generation in subunit form for use as cancer vaccines, whole tumor cells remain a potent vehicle for generating anti-tumor immunity. This is because tumor cells express an array of target antigens for the immune system to react against, avoiding problems associated with major histocompatibility complex (MHC)-restricted epitope identification for individual patients. Furthermore, whole cells are relatively simple to propagate and are potentially efficient at contributing to the process of T cell priming. However, whole cells can also possess properties that allow for immune evasion, and so the question remains of how to enhance the immune response against tumor cells so that they are rejected. Scenarios where whole tumor cells may be utilised in immunotherapy include autologous tumor cell vaccines generated from resected primary tumor, allogeneic (MHC-disparate) cross-reactive tumor cell line vaccines, and immunotherapy of tumors in situ. Since tumor cells are considered poorly immunogenic, mainly because they express self-antigens in a non-stimulatory context, the environment of the tumor cells may have to be modified to become stimulatory by using immunological adjuvants. Recent studies have re-evaluated the relative roles of direct and cross-priming in generating anti-tumor immunity and have highlighted the need to circumvent immune evasion [133].

The Wilms tumor gene WT1 is expressed in leukemias and various kinds of solid tumors, including lung and breast cancer, and exerts an oncogenic function in these malignancies, suggesting that WT1 protein is a novel, overexpressed tumor antigen. The WT1 protein, in fact, is an attractive tumor rejection antigen in animal models. Stimulation in vitro of peripheral blood mononuclear cells with HLA-A*2402--and HLA-A*0201--restricted 9-mer WT1 peptides

elicits WT1-specific cytotoxic T-lymphocytes (CTLs), and the CTLs kill endogenously WT1-expressing leukemia or solid tumor cells. Furthermore, WT1 immunoglobulin M (IgM) and IgG antibodies are detected in patients with hematopoietic malignancies such as acute myeloid leukemia, chronic myeloid leukemia, and myelodysplastic syndromes, indicating that WT1 protein overexpressed by leukemia cells is indeed immunogenic. Taken together, these results demonstrate that WT1 protein is a promising tumor antigen for cancer immunotherapy against leukemias and various kinds of solid tumors, including lung and breast cancer [134].

In the last few years, a great deal of efforts have been directed towards understanding the molecular basis of T cell-mediated anti-tumor immunity and elucidating the molecular nature of tumor antigens recognized by T cells. Identification of a number of major histocompatibility complex (MHC) class I-restricted melanoma antigens has led to clinical trials aimed at developing effective cancer vaccines. These studies showed some evidence of therapeutic effect on the treatment of cancer, but the exclusive use of CD8+ T cells may not be effective in eradicating tumor. This rekindles interest in the role of CD4+ T cells in antitumor immunity, which play a central role in orchestrating the host immune response against cancer. Thus, Wang *et al*. [135] have attempted to identify MHC class II-restricted tumor antigens recognized by tumor-specific CD4+ T cells. The identification of tumor rejection antigens provides new opportunities for the development of therapeutic strategies against cancer.

Interleukin (IL)-2 and IL-15 are two cytokine growth factors that regulate lymphocyte function and homeostasis. Early clinical interest in the use of IL-2 in the immunotherapy of renal cell carcinoma and malignant melanoma demonstrated the first efficacy for cytokine monotherapy in the treatment of neoplastic disease. Advances in our understanding of the cellular and molecular biology of IL-2 and its receptor complex have provided rationale to better utilize IL-2 to expand and activate immune effectors in patients with cancer. Exciting new developments in monoclonal antibodies recognizing tumor targets and tumor vaccines have provided new avenues to combine with IL-2 therapy in cancer patients. IL-15, initially thought to mediate similar biological effects as IL-2, has been shown to have unique properties in basic and pre-clinical studies that may be of benefit in the immunotherapy of cancer [136].

Several recent developments have hallmarked progress in tumor immunology and immunotherapy. The use of interleukin-2 (IL-2) in cancer patients demonstrated that an immunological manipulation was capable of mediating the regression of established growing cancers in humans. The identification of the genes encoding cancer antigens and the development of means for effectively immunizing patients against these antigens has opened important new avenues of exploration for the development of effective active and cell-transfer immunotherapies for patients with cancer [137].

A wide range of strategies in cancer immunotherapy has been developed in the last decade, some of which are currently being used in clinical settings. The development of these immunotherapeutical strategies has been facilitated by the generation of relevant transgenic animal models. Since the different strategies in experimental immunotherapy of cancer each aim to activate different immune system components, a variety of transgenic animals have been generated either expressing tumor associated, HLA, oncogenic or immune effector cell molecule proteins [138].

Immunotherapy is in its infancy for many diseases, whether they are neoplastic or autoimmune. The major issues for cancer immunotherapy today involve the definition of molecular targets and the generation of effector mechanisms to attack the targets of interest. Soft tissue sarcomas provide a unique opportunity to examine the immune response against defined antigens. Many types of sarcomas contain tumor-specific chromosomal translocations encoding fusion proteins, which are attractive targets for immunotherapy. Our understanding of the immune system is also coming into clearer focus with the discovery of dendritic cells as powerful natural adjuvants and the teasing out of mechanisms leading to immunity versus tolerance as examples. It is hoped that the intersection of cellular immunology and sarcoma molecular biology will lead to new modalities of therapy for this group of patients with this heterogeneous group of diseases [139].

Studies of the administration of interleukin-2 to patients with metastatic melanoma or kidney cancer have shown that immunological manipulations can mediate the durable

regression of metastatic cancer. The molecular identification of cancer antigens has opened new possibilities for the development of effective immunotherapies for patients with cancer. Clinical studies using immunization with peptides derived from cancer antigens have shown that high levels of lymphocytes with anti-tumor activity can be raised in cancer-bearing patients. Highly avid anti-tumor lymphocytes can be isolated from immunized patients and grown in vitro for use in cell-transfer therapies. Current studies are aimed at understanding the mechanisms that enable the cancer to escape from immune attack [140].

The idea that there might be an immune response to cancer has been around for many years. Immunotherapy has a long history, but is only rarely considered as the treatment of choice. Immunotherapy has encountered a number of intrinsic difficulties in cancer, such as the antigenic resemblance between the tumor and normal cells, the rapid kinetic proliferation of tumor cells and their reduced immunogenicity. There are various types of immunotherapy. Aspecific immunotherapy augments the body's immune response without targeting specific tumoral antigens. In adoptive immunotherapy, cells are administered with antitumoral reactivity to mediate neoplasm regression. Specific active immunotherapy is based on the principle that neoplasm cells contain immunogenic sites against which an antitumoral immune response can be induced in an attempt to stimulate the immune system to target specific tumoral antigens. Vaccines against cancer cells are based on a more precise identification of the tumoral antigen components. Passive immunotherapy was limited by the difficulty of obtaining high titering and specificity in early attempts using polyclonal antisera; monoclonal antibodies are currently used alone or in association with radioactive substances and cytotoxic agents. Enormous progress has been made this century in the use of immunotherapy for cancer treatment. It seems likely that the next century will see its increased afficacy, making it one of the possible therapeutic options [141].

Despite major advances in our understanding of adaptive immunity and dendritic cells, consistent and durable responses to cancer vaccines remain elusive and active immunotherapy is still not an established treatment modality. The key to developing an effective anti-tumor response is understanding why, initially, the immune system is unable to detect transformed cells and is subsequently tolerant of tumor growth and metastasis. Ineffective antigen presentation limits the adaptive immune response; however, we are now learning that the host's innate immune system may first fail to recognize the tumor as posing a danger. Recent descriptions of stress-induced ligands on tumor cells recognized by innate effector cells, new subsets of T cells that regulate tumor tolerance and the development of spontaneous tumors in mice that lack immune effector molecules, beckon a reflection on our current perspectives on the interaction of transformed cells with the immune system and offer new hope of stimulating therapeutic immunity to cancer [142].

Immunotherapy approaches to fight cancer are based on the principle of mounting an immune response against a self-antigen expressed by the tumor cells. In order to reduce potential autoimmunity side-effects, the antigens used should be as tumor-specific as possible. A complementary approach to experimental tumor antigen discovery is to screen the human genome in silico, particularly the databases of "Expressed Sequence Tags" (ESTs), in search of tumor-specific and tumor-associated antigens. The public databases currently provide a massive amount of ESTs from several hundreds of cDNA tissue libraries, including tumoral tissues from various types. Vinals *et al*. [143] described a novel method of EST database screening that allows new potential tumor-associated genes to be efficiently selected. The resulting list of candidates is enriched in known genes, described as being expressed in tumor cells.

The concept of immunotherapy of cancer is more than a century old, but only recently have molecularly defined therapeutic approaches been developed. The identification of tumor antigens can now be accelerated by methods allowing the amplification of gene products selectively or preferentially transcribed in the tumor. However, determining the potential immunogenicity of such gene products remains a demanding task, since major histocompatibility complex (MHC) restriction of T cells implies that for any newly defined antigen, immunogenicity will have to be defined for any individual MHC haplotype. Tumor-derived peptides eluted from MHC molecules of tumor tissue are also a promising source of antigen. Tumor antigens are mostly of weak immunogenicity, because the vast majority is tumor-associated differentiation antigens already 'seen' by the patient's immune

system. Effective therapeutic vaccination will thus require adjuvant support, possibly by new approaches to immunomodulation such as bispecific antibodies or antibody-cytokine fusion proteins. Tumor-specific antigens, which could be a more potent target for immunotherapy, mostly arise by point mutations and have the disadvantage of being not only tumor-specific, but also individual-specific. Therapeutic vaccination will probably focus on defined antigens offered as protein, peptide or nucleic acid. Irrespective of the form in which the antigen is applied, emphasis will be given to the activation of dendritic cells as professional antigen presenters. Dendritic cells may be loaded in vitro with antigen, or, alternatively, initiation of an immune response may be approached in vivo by vaccination with RNA or DNA, given as such or packed into attenuated bacteria. The importance of activation of T helper cells has only recently been taken into account in cancer vaccination. Activation of cytotoxic T cells is facilitated by the provision of T helper cell-derived cytokines. T helper cell-dependent recruitment of elements of non-adaptive defence, such as leucocytes, natural killer cells and monocytes, is of particular importance when the tumor has lost MHC class I expression. Barriers to successful therapeutic vaccination include: (i) the escape mechanisms developed by tumor cells in response to immune attack; (ii) tolerance or anergy of the evoked immune response; (iii) the theoretical possibility of provoking an autoimmune reaction by vaccination against tumor-associated antigens; and (iv) the advanced age of many patients, implying reduced responsiveness of the senescent immune system [144].

Generating an antitumor immune response in tumor-bearing host has been impaired by several characteristics of both patient and tumor cells. Amongst those requirements is the necessity of generating immune effectors that are specific to tumor cells. The last two decades have seen the description of many so called tumor "specific" antigens. Indeed, strictly specific tumor antigens are scarce. Most antigens are tumor-associated antigens, also shared by normal tissues. Telomerase and its activity have recently been recognized as a major marker of tumoral growth in more than 80% of cancers. Some telomerase subunits might be ideal, if not universal, targets to an antitumor immune response in patients with cancer. Many other major parameters remain to be understood and to be mastered [145].

The survival of patients with cancer has improved steadily but incrementally over the last century, with the advent of effective anticancer treatments such as chemotherapy and radiotherapy. However, the majority of patients with metastatic disease will not be cured by these measures and will eventually die of their disease. New and more effective methods of treating these patients are required urgently. The immune system is a potent force for rejecting transplanted organs or microbial pathogens, but effective spontaneous immunologically induced cancer remissions are very rare. In recent years, much has been discovered about the mechanisms by which the immune system recognizes and responds to cancers. The specific antigens involved have now been defined in many cases. Improved adjuvants are available. Means by which cancer cells overcome immunological attack can be exploited and overcome. Most importantly, the immunological control mechanisms responsible for initiating and maintaining an effective immune response are now much better understood. It is now possible to manipulate immunological effector cells or antigen-presenting cells ex vivo in order to induce an effective antitumour response. At the same time, it is possible to recruit other aspects of the immune system, both specific (e.g. antibody responses) and innate (natural killer cells and granulocytes) [146].

Immunotherapy of cancer is entering into a new phase of active investigation both at the pre-clinical and clinical level. This is due to the exciting developments in basic immunology and tumor biology that have allowed a tremendous increase in our understanding of mechanisms of interactions between the immune system and tumor cells. Clinical approaches are diverse but can now be based on strong scientific rationales. The analysis of the available clinical results suggests that, despite some disappointments, there is room for optimism that both active immunotherapy (vaccination) and adoptive immunotherapy may soon become part of the therapeutic arsenal to combat cancer in a more efficient way [147].

Advancements in the understanding of cellular immunity within the last decade, along with the characterization of tumor antigens, have led to immunotherapeutic approaches for cancer therapy. This mode of treatment is expected to provide more tumor-specific activity, thereby being less toxic to normal cells than standard modalities. Clinical trials are underway

throughout the world to determine whether immunotherapy is a practical and viable alternative to conventional cancer therapies. Unlike radiotherapy and chemotherapy, wherein tumor regression is the standard for determining efficacy of the regimens, immunotherapy has to be evaluated by the examination of several immunological parameters within patients [148].

The identification of tumor-associated antigens recognized by cellular or humoral effectors of the immune system has opened new perspectives for cancer therapy. Different groups of cancer-associated antigens have been described as targets for cytotoxic T lymphocytes (CTLs) in vitro and in vivo: 1) cancer-testis (CT) antigens, which are expressed in different tumors and normal testis; 2) melanocyte differentiation antigens; 3) point mutations of normal genes; 4) antigens that are overexpressed in malignant tissues; and 5) viral antigens. Clinical studies with peptides derived from these antigens have been initiated to induce specific CTL responses in vivo. Immunological and clinical parameters for the assessment of peptide-specific reactions have been defined, i.e., delayed-type hypersensitivity (DTH), CTL, autoimmmune, and tumor regression responses. Preliminary results demonstrate that tumor-associated peptides alone elicit specific DTH and CTL responses leading to tumor regression after intradermal injection. Granulocyte-macrophage colony-stimulating factor (GM-CSF) was proven effective in enhancing peptide-specific immune reactions by amplification of dermal peptide-presenting dendritic cells. Long-lasting complete tumor regressions have been observed after induction of peptide-specific CTLs. However, in single cases with disease progression after an initial tumor response, either a loss of the respective tumor antigen targeted by CTLs or of the presenting major histocompatibility complex (MHC) class I allele was detected as a mechanism of immune escape under immunization. Based on these observations, cytokines to enhance antigen and MHC class I expression in vivo are being evaluated to prevent immunoselection. Recently, a strategy utilizing spontaneous antibody responses to tumor-associated antigens (SEREX) has led to the identification of a new CT antigen, NY-ESO-1, which is regarded as one of the most immunogenic antigens known today inducing spontaneous immune responses in 50% of patients with NY-ESO-1-expressing cancers. Clinical studies involving antigenic constructs that induce both antibody and CTL responses will show whether these are more effective for immunotherapy of cancer [149].

Tumors express proteins not commonly found in normal cells, or over-express certain proteins. These may in some cases serve as target antigens for immunological attack. It is therefore essential to improve our understanding of the nature of these target epitopes and the cells which recognize them, in order to develop immunotherapy as a realistic treatment for cancer [150].

Advances in molecular biology have enabled specific antigens present on colorectal cells to be characterized, against which immune responses may be generated. This, in combination with our inability to significantly alter survival from this condition, has resurrected an interest in immunotherapy as a potential treatment option. A number of approaches currently constitute immunotherapeutic options for colorectal cancer. A number of treatment modalities are already in phase III studies, although clearly not all will fulfill their initial promise. Surgeons need to be aware of the advances in this rapidly expanding field, and keep an open mind as to their efficacy [151].

The goal of harnessing the immune system to recognize tumor as "nonself" is not new. Now, thanks to new knowledge and new techniques, however, modalities that seek to activate the host immune system are becoming increasingly feasible as treatments for advanced malignancies [152].

The major impact of recent scientific advances, such as the discovery of genes and gene products, has been to facilitate development of immunotherapies based on the specific stimulation of immune reactions against characterized tumor antigens [153].

Over the last decade, there has been a considerable increase in understanding of immune responses against cancers, the antigenic structures on tumor cells recognised by the immune system, and the development of more effective vaccines. There is, however, very limited understanding of why the immune system most often fails to control tumor growth and progression. In some patients, it is difficult to demonstrate immune responses to their tumors, and it may be assumed that this reflects poor recognition of tumor antigens, induction of anergy in lymphocytes, or suppression of

immune responses by tumor-derived factors. In other patients, tumor progression appears to occur despite the presence of antibody or cell-mediated responses. This may indicate selection of tumor cells that have lost tumor antigens or HLA antigens by immune responses against the tumor. Tumor cells may also become resistant to mediators of apoptosis, such as Fas ligand and tumor necrosis factor-related apoptosis-inducing ligand used by lymphocytes to kill tumor cells. It is suggested that development of effective immunotherapy will need to include strategies that take into account these limitations of immune responses and classification of tumors according to the treatment approach most likely to succeed [154].

Heat shock proteins (Hsps), ubiquitous in nature, act as chaperones for peptides and other proteins. They have been implicated in loading immunogenic peptides onto major histocompatibility complex molecules for presentation to T cells. When isolated from tumor cells, Hsps are complexed with a wide array of peptides, some of which serve as tumor-specific antigens. Animal studies have demonstrated that heat shock protein--peptide complexes (HSPPCs) from tumor cells can act as vaccines to prevent or treat tumors. Potent and specific tumor antigens have long been the holy grail in cancer immunotherapy; HSPPCs from tumor cells could become a safe and reliable source of tumor-specific antigens for clinical application [155].

Immunotherapy of mice with preexisting cancers with heat shock protein preparations derived from autologous cancer resulted in retarded progression of the primary cancer, a reduced metastatic load, and prolongation of life-span. Treatment with heat shock protein preparations derived from cancers other than the autologous cancer did not provide significant protection. Spontaneous cancers (lung cancer and melanoma), chemically induced cancers (fibrosarcoma and colon carcinoma), and an ultraviolet radiation-induced spindle cell carcinoma were tested, and the results support the efficacy of autologous cancer-derived heat shock protein-peptide complexes in immunotherapy of cancers without the need to identify specific tumor antigenic epitopes [156].

9. DENDRITIC CELLS

The identification of tumor specific antigens has provided important advance in tumor immunology. It is now established that specific cytotoxic T lymphocytes (CTL) and natural killer cells infiltrate tumor tissues and are effector cells able to control tumor growth. However, such a natural antitumor immunity has limited effects in cancer patients. Failure of host defenses against tumor is consecutive to several mechanisms which are becoming targets to design new immunotherapeutic approaches. CTL are critical components of the immune response to human tumors and induction of strong CTL responses is the goal of most current vaccine strategies. Effectiveness of cytokine therapy, cancer vaccines and injection of cells improving cellular immunity have been established in tumor grafted murine models. Clinical trials are underway. Today, interest is particularly focused on cell therapy: injected cells are either "ready to use" effector cells (lymphocytes) or antigen presenting cells able to induce a protective immune reaction in vivo (dendritic cells). The challenge ahead lie in the careful optimization of the most promising strategies in clinical situation [157].

The response of hepatocellular carcinoma (HCC) to therapy is often disappointing and new modalities of treatment are clearly needed. Active immunotherapy based on the injection of autologous dendritic cells (DC) co-cultured ex vivo with tumor antigens has been used in pilot studies in various malignancies such as melanoma and lymphoma with encouraging results. In the study of Ladhams et al. [158], the preparation and exposure of patient DC to autologous HCC antigens and re-injection in an attempt to elicit antitumor immune responses were described. Therapy was given to two patients, one with hepatitis C and one with hepatitis B, who had large, multiple HCC and for whom no other therapy was available. No significant side-effects were observed. The clinical course was unchanged in one patient, who died a few months later. The other patient, whose initial prognosis was considered poor, is still alive and well more than 3 years later with evidence of slowing of tumor growth based on organ imaging.

The characterization of tumor-associated antigens recognized by human T lymphocytes in a major histocompatibility complex (MHC)-restricted fashion has opened new possibilities for immunotherapeutic approaches to the treatment of human cancers. Dendritic cells (DC) are professional antigen presenting cells that are well suited to activate T cells toward various

antigens, such as tumor-associated antigens, due to their potent costimulatory activity. The availability of large numbers of DC, generated either from hematopoietic progenitor cells or monocytes in vitro or isolated from peripheral blood, has profoundly changed pre-clinical research as well as the clinical evaluation of these cells. Accordingly, appropriately pulsed or transfected DC may be used for vaccination in the field of infectious diseases or tumor immunotherapy to induce antigen-specific T cell responses. These observations led to pilot clinical trials of DC vaccination for patients with cancer in order to investigate the feasibility, safety, as well as the immunologic and clinical effects of this approach. Initial clinical studies of human DC vaccines are generating encouraging preliminary results demonstrating induction of tumor-specific immune responses and tumor regression. Nevertheless, much work is still needed to address several variables that are critical for optimizing this approach and to determine the role of DC-based vaccines in tumor immunotherapy [159].

Dendritic cells are among the most efficient antigen-presenting cells of our immune system and they play a crucial role in immunity reactions such as the activation of T and B cells and the induction or maintenance of tolerance. New culture methods allow us to generate dendritic cells in sufficient numbers for further studies and for the preparation of antigen-loaded dendritic cells for clinical application in cancer patients. In animal studies immunization with antigen-loaded dendritic cells offered protection from growth of injected tumor cells. In experimental clinical studies in cancer patients with e.g. metastatic renal carcinoma, melanoma and B cell lymphoma some lasting remissions were observed after administration of antigen-loaded dendritic cells. Side effects were minor. Unanswered questions on tumor vaccines with antigen-loaded dendritic cells concern specific matters, such as optimal culture methods and antigen loading, and general matters, such as dose, frequency, duration and route of administration. Also, no method is currently available by which the in vivo immune response can be measured accurately [160].

Research over the last two years has explored a number of possible approaches to applying dendritic cell immunotherapy to the treatment of human cancers. The chosen strategy in clinical situations will vary for individual patients and will depend on the type of tumor, availability of tissue samples and potential source of dendritic cells. The isolation of fractionated tumor peptide from individual patients' tumors for use with autologous stem cell-derived dendritic cells may provide, in at least some cases, an effective and practical approach to cancer immunotherapy. This approach will provide a 'closed' system of tumor immunotherapy with all components (dendritic cells, antigen and cytotoxic T lymphocytes) being provided by the patient and applied in a tailor-made fashion to individual patients as an adjuvant to current anti-tumor therapies [161].

10. CANCER ANTIBODIES

The specificity of antibodies has been harnessed to target cancer cells and the first therapeutic antibodies for use in oncology are now finding application in the clinic. Studies are currently under way to develop new and improved antibodies. Recent developments have been made in the identification of novel targets, including the use of genomic and proteomic technologies. Several methods are also being developed to enhance antibody efficacy [162].

Bi-specific antibodies (BsAbs) combine immune cell activation with tumor cell recognition as a result of which tumor cells are killed by pre-defined effector cells [163].

Antibody-based therapy of human cancers has led to several remarkable outcomes, particularly in the therapy of breast cancer and lymphoma. Many solid tumors have proven less responsive, due in part to difficulties in the tumor-selective delivery of antibodies and potential cytolytic effectors. However, antibodies that directly perturb signaling mechanisms in cells derived from epithelial malignancies have shown benefit; examples include antibodies directed against the extracellular domains of HER2/neu and epidermal growth factor receptor. A long-term goal of immunotherapy has been to induce anti-tumor inflammatory responses that can directly cause tumor regression or induce adaptive responses against tumor-related antigens [164].

Specific targeting of tumor cells may be achieved by using monoclonal antibodies to tumor antigens. Edrecolomab is a mouse-derived monoclonal IGg2A antibody directed against the human tumor-associated CO17-1A (or Ep-CAM) antigen, and is the first monoclonal antibody approved for cancer therapy. Encouraging results of several clinical trials were

recently reported using edrecolomab for adjuvant therapy after surgery of Duke's C colorectal cancer. Side effects and toxicity profiles compare favorably to conventional regimens of radio- or chemotherapy. Future challenges lie in further improvement of these novel therapeutics, hopefully generating benefit for a larger number of cancer patients [165].

Gangliosides on tumor cells have been suggested as potential target antigens for specific immunotherapy in various types of cancer including small cell lung cancer (SCLC). Brezicka et al. [166] have compared the expression of three gangliosides that have been described as tumor-associated antigens, FucGM1, GM2 and GD3 in SCLC tissue specimens collected at autopsy, using a double-layer immunofluorescence staining method and specific monoclonal antibodies (Mabs) directed against these ganliosides. They found expression of FucGM1, GD3 and GM2 in 70% (n=20), 60% (n=15) and 40% of the tumor cells in all lesions from the same patient (five of eight cases). These results indicate that FucGM1 is a relevant ganglioside antigen in SCLC, and suggest that specific immunotherapy involving more than one ganglioside antigen in SCLC should at least include FucGM1 and GD3.

There is now a considerable body of information documenting the autoimmune consequences of antibodies induced by growing malignancies, or by passively administered and actively induced antibodies, in cancer patients against antigens shared by normal and malignant tissues. This provides a rich source of information addressing the consequences of autoantibodies against a range of antigens. Antibodies against cell-surface or intracellular antigens in the central nervous system (CNS) or on epithelial surfaces of normal tissues do not generally result in autoimmunity, but the same types and titers of antibodies against cell surface antigens in the subepidermal skin, peripheral nerves, blood, or vascular sites such as the spleen and bone marrow readily induce autoimmunity. The blood brain barrier of the CNS and apical antigen expression and the basement membrane in epithelial tissues, may protect these sites from antibody induced damage. Cancer cells, however, are protected by neither unidirectional antigen expression nor basement membranes. Vaccine induced antibodies against a variety of cancer cell surface antigens have been associated with prevention of tumor recurrence in preclinical models and in vaccinated cancer patients, in the absence of demonstrable autoimmunity. This forms the basis for a series of ongoing Phase III trials with single or polyvalent antigen cancer vaccines designed for optimal antibody induction [167].

Immunotherapy of cancer is still mainly an experimental treatment. Some monoclonal antibodies have been approved for adjuvant therapy of cancer in patients, but active immunization strategies have not yet matured to this stage. The fact that vaccination against viral diseases is effective has primed high expectations for successful vaccination against cancer as well. Indeed, in some animal models, therapeutic results could be obtained against short-term established tumors, which paved the way for clinical trials. However, the first results with active immunization in cancer patients were disappointing and this led to a careful examination of current protocols and the search for more effective approaches. Evaluation of the available data suggests that cancer patients may not be comparable in their immune response to cancer vaccines with healthy persons. Furthermore, the tumor seems to be able to develop several immune-escape mechanisms, which either inactivate the specific immune cells or prevent activation of potential effector mechanisms against the tumor [168].

11. GENETIC IMMUNOTHERAPY

The establishment of cancer in a host involves at least two major events: the escape of tumor cells from normal growth control and their escape from immunological recognition. Because of this nature of their development, cancer cells seem to be predominatly poorly immunogenic. In contrast to the previous idea that cancer cells express no recognizable antigens, recent progress in the identification and characterization of tumor antigens, as well as the expansion of knowledge on the cellular and molecular mechanisms of antigen recognition by the immune system, have raised the possibility of using immunotherapy to treat certain tumors. Information on these mechanisms has been obtained in three crucial areas: 1) the role of cytokines in the regulation of the immune response, 2) the molecular characterization of tumor antigens in both mouse and human tumors, and 3) the molecular mechanisms of T cell activation and antigen presentation. Such information has provided new insight into tumor immunology and immunotherapy. Furthermore, recombinant DNA technology allows for

modification of the genome of mammalian cells for therapeutic purposes in several diseases. Several novel strategies have been developed to derive genetically modified tumor cells and use them as cellular vaccines to induce antitumor immunity in animal tumor models. This combined modality of genetically modified tumor cells and immunotherapy has been termed immunogene therapy of tumors. Crucial to this approach has been the ability to transfer into normal or neoplastic cells genes known to increase the immunogenicity of cells, which subsequently can be used to augment immune reactions in tumor-bearing mice or cancer patients. While there has been success in inducing antitumor immunity in some tumor models, there are difficulties and limitations in the application of these gene-modified tumor cells for the treatment of preexisting tumors [169].

Genetic immunization refers to treatment strategies where gene transfer methods are used to generate immune responses against cancer. Our growing knowledge of the mechanisms regulating the initiation and maintenance of cytotoxic immune responses has provided the rationale for the design of several genetic immunization strategies. Tumor cells have been gene-modified to express immune stimulatory genes and are then administered as tumor vaccines, in an attempt to overcome tumor cell ignorance by the immune system. With the description of well-characterized tumor antigens, multiple strategies have been proposed mainly aimed at optimal tumor antigen presentation by antigen-presenting cells (APC). Among APC, the dendritic cells have been recognized as the most powerful cells in this class, and have become the target for introducing tumor antigen genes to initiate antitumor immune responses. The detailed knowledge of how the immune system can be activated to specifically recognize tumor antigens, and the mechanisms involved in the control of this immune response, provide the basis for modern genetic immunization strategies for cancer treatment [170].

T lymphocytes play a crucial role in the host's immune response to cancer. Although there is ample evidence for the presence of tumor-associated antigens on a variety of tumors, they are seemingly unable to elicit an adequate antitumor immune response. Modern cancer immunotherapies are therefore designed to induce or enhance T cell reactivity against tumor antigens. Vaccines consisting of tumor cells transduced with cytokine genes in order to enhance their immunogenicity have been intensely investigated in the past decade and are currently being tested in clinical trials. With the development of novel gene transfer technologies it has now become possible to transfer cytokine genes directly into tumors in vivo. The identification of genes encoding tumor-associated antigens and their peptide products which are recognized by cytotoxic T lymphocytes in the context of major histocompatibility complex class I molecules has allowed development of DNA-based vaccines against defined tumor antigens. Recombinant viral vectors expressing model tumor antigens have shown promising results in experimental models. This has led to clinical trials with replication-defective adenoviruses encoding melanoma-associated antigens for the treatment of patients with melanoma. An attractive alternative concept is the use of plasmid DNA, which can elicit both humoral and cellular immune responses following injection into muscle or skin. New insights into the molecular biology of antigen processing and presentation have revealed the importance of dendritic cells for the induction of primary antigen-specific T cell responses. Considerable clinical interest has arisen to employ dendritic cells as a vehicle to induce tumor antigen-specific immunity. Advances in culture techniques have allowed the generation of large numbers of immunostimulatory dendritic cells in vitro from precursor populations derived from blood or bone marrow. Experimental immunotherapies which now transfer genes encoding tumor-associated antigens or cytokines directly into professional antigen-presenting cells such as dendritic cells are under evaluation in pre-clinical studies at many centers. Gene therapy strategies, such as in vivo cytokine gene transfer directly into tumors as well as the introduction of genes encoding tumor-associated antigens into antigen-presenting cells hold considerable promise for the treatment of patients with cancer [171].

12. ADJUVANT IMMUNOTHERAPY

In the course of a century, tumor immunology has revealed a picture of a very complex immune system involving the recognition and eradication of malignancies. Many tumors evade the immune system, and understanding of tumor escape mechanisms is the key to a successful immunotherapy for cancer. A wide array of tumor immunotherapy modalities have been developed, many of which have reached the

phase of clinical trials, with some satisfactory results [172].

Although surgery remains the mainstay for the treatment of most solid tumors, investigators are seeking complementary therapies to eradicate microscopic disease, which causes tumor relapse even after an apparently complete surgical excision. Although adjuvant chemotherapy has achieved some significant results, the control of minimal residual disease is still a challenge for clinicians. Among novel therapeutic approaches, immunotherapy holds promise. This anticancer strategy aims at triggering a highly specific endogenous killing machine against tumor cells. Recent progress in tumor immunology has improved our understanding of host-immune system interactions. In particular, new technologies have fostered the identification of potentially immunogenic tumor antigens that can be used as suitable targets for immune effector cells. After observing immunotherapy-mediated clinical responses in patients with metastatic disease, investigators have started evaluating this anticancer modality in the adjuvant setting [173].

13. INTRALIPID EFFECTS ON THE IMMUNE SYSTEM

Intravenous lipid emulsions have been used experimentally since at least the 19th century. An early product marketed in 1957 under the name Lipomul was briefly used in the United States but was subsequently withdrawn due to side effects. Intralipid was invented by the Swedish physician and nutrition researcher Arvid Wretlind [175], and was approved for clinical use in Sweden in 1962. In the United States, the Food and Drug Administration initially declined to approve the product due to prior experience with another fat emulsion. It was approved in the United States in 1972.

Recurrent embryo implantation failure (RIF) is a disorder with potentially devastating physiological and psychological manifestations for those affected. Although its prevalence is not uncommon, many of the mechanisms involved still require elucidation. Both organ-specific and systemic autoimmunity are associated with an increased prevalence of recurrent miscarriage and reproductive failure, rendering the role of the maternal immunological system in fertility a key concept. It is believed by some that central to this theme is the maternal cytokine profile, with particularly T-helper (Th) cells. Immune modulating therapies have therefore been mooted as potential therapeutic strategies. Recent reports of high pregnancy rates achievable in women with RIF have added fuel to the debate regarding the effectiveness of intralipid in modulating the immune system. We would like to assess if there is sufficient current evidence of acceptable quality to permit an assumption that intralipid therapy is an effective treatment for women undergoing repeated assisted reproduction cycles. We have concluded that appropriately controlled, large-scale, confirmatory studies are necessary to prove the efficacy of intralipid before it can be recommended for routine use [176].

In vitro investigations have revealed the ability of intralipids to suppress natural killer (NK) cytotoxicity. Evidence from both animal and human studies suggests that intralipid administered intravenously may enhance implantation and maintenance of pregnancy when the patient has an abnormal NK cell level or function.

The aim of this study was to establish the duration and efficacy of Intralipids suppressive effect on NK cell functional activity.

Fifty patients with abnormal NK activity results (NKa) received intralipid 20% i.v. (9 mg/mL total blood volume -corresponds to 2 mL of intralipid 20% diluted in 250 mL saline; or 18 mg/mL - corresponds to 4 mL of intralipid 20% diluted in 250 mL saline) infusions and their NKa were tested periodically. The determination of NK cell function was performed by flow cytometry using K562 cells as targets.

Fifty women with abnormal NKa-testing received intralipid infusions. 39 (78%) showed NKa suppression within the normal range the first week after infusion, 11 (22%), showed suppression, but still above the normal threshold. They received second infusion 2-3 weeks later. In 10, the Nka activity was normalized the following week. Four patients had three intralipid infusions in 2-week periods in between and after the third infusion, and all showed NKa normal activity. In 47 patients the suppressive effect of the Intralipid after the normalization of NKa lasted between 6 and 9 weeks, in two patients this benefit lasted 5 weeks, and in one patient the effect was 4 weeks.

Intralipid is effective in suppressing in vivo abnormal NK-cell functional activity. The results suggest that Intralipid can be used successfully as a therapeutic option to modulate abnormal NK activity in women with reproductive failure [177].

Novel anti-inflammatory effects of insulin have recently been described, and insulin therapy to maintain euglycemia suppresses the plasma levels of free fatty acids (FFA) and increases the survival of critically ill patients. We aimed to explore the effect of short-term high levels of plasma FFA on the inflammatory response to a low dose of endotoxin. Fourteen healthy male volunteers underwent the following two trials in a randomized crossover design: 1) continuous infusion of 20% Intralipid [0.7 ml.kg(-1).h(-1) (1.54 g/kg)] for 11 h, and 2) infusion of isotonic saline for 11 h (control). In each trial, heparin was given to activate lipoprotein lipase, and an intravenous bolus of endotoxin (0.1 ng/kg) was given after 6 h of Intralipid/saline infusion. Blood samples and muscle and fat biopsies were obtained before the Intralipid/saline infusion and before as well as after infusion of an endotoxin bolus. Plasma levels of FFA, triglycerides, and glycerol were markedly increased during the Intralipid infusion. Endotoxin exposure induced an increase in plasma levels of TNF-alpha, IL-6, and neutrophils and further stimulated gene expression of TNF-alpha and IL-6 in both skeletal muscle and adipose tissue. The systemic inflammatory response to endotoxin was significantly pronounced during Intralipid infusion. Short-term hyperlipidemia enhances the inflammatory response to endotoxin, and skeletal muscle and adipose tissue are capable of producing essential inflammatory mediators after endotoxin stimulation [178].

Because of its oxidative modification during the acute-phase response to an aggression, low density lipoprotein (LDL) can be regarded as a source of lipid mediators that can act both to promote and inhibit inflammation. This can be exemplified by the production of anti-inflammatory oxidized fatty acids and proinflammatory lysophosphatidylcholine (LPC) during LDL oxidation. We have shown previously that oxidized LDL (oxLDL) plays an active role at the interface between innate and adaptive immunity by delivering instructive molecules such as LPC, which promotes mature dendritic cell (DC) generation from differentiating monocytes. It is shown in this study that LPC affects the signaling pathway of peroxisome proliferator-activated receptors (PPARs). LPC-induced DC maturation is associated with complete inhibition of PPARgamma activity and up-regulation of the activity of an uncharacterized nuclear receptor that bind peroxisome proliferator response element. Oxidized fatty acids generated during LDL oxidation are natural ligands for PPARgamma and inhibit oxLDL- and LPC-induced maturation. Inhibition experiments with synthetic PPARgamma ligands suggested a PPARgamma-dependent and independent effect of LPC on DC maturation. Therefore, the relative amount of oxidized fatty acids and LPC influences the immunological functions of oxLDL on DC, in part by regulating the PPAR pathway. By sensing the biochemical composition of lipoprotein particles, the innate immune system may thus identify various endogenous signals that influence the immune response during the acute-phase reaction. The therapeutic emulsion intralipid also blocks LPC action on PPAR activity and DC maturation. Intralipid may thus be an alternative therapeutic strategy for some chronic inflammatory diseases [179].

During the acute phase response, the interplay between high density lipoproteins and low density lipoproteins (LDL) favors transient generation of oxidized LDL with proinflammatory activities. We hypothesized that oxidative modification of LDL is an endogenous signal for the immune system, and we have shown that oxidized LDL promotes mature dendritic cell transition from monocyte, therefore linking the nonspecific acute phase response to adaptive immunity. Lysophosphatidylcholine (LPC) is a major lipid component of oxidized LDL with reported proinflammatory activities. We now report that LPC acts through G protein-coupled receptors on differentiating monocytes to generate mature dendritic cells with the ability to stimulate IL-2 and IFN-gamma production by allogeneic T lymphocytes. LPC is most effective in lipoprotein-deprived serum and can be inhibited by an excess of native LDLs reflecting normal plasma conditions. Therefore, by controlling the balance between native and oxidized lipoproteins and the resulting production of LPC, the acute phase reactants may provide a context of Ag presentation that is transiently favorable to immune activation. Intralipid, a therapeutic lipid emulsion for parenteral nutrition with unexplained immunomodulatory properties, also blocked LPC activity. This opens perspectives for the understanding and

treatment of acute and chronic inflammatory diseases [180].

The lipid component of total parenteral nutrition (TPN) has reportedly been associated with trophic effects on the intestinal mucosa and suppressive effects on the immune system.

We have challenged these hypotheses using a 7-day TPN rodent model comparing the effects of isocaloric, isonitrogenous lipid-based (TPN-lipid, 50% of calories as long-chain triacylglycerol) and carbohydrate-based TPN (TPN-CH, 100% of calories as carbohydrates) on mucosal morphology and immune function. Enterally fed animals were included to establish a baseline for immunologic read-outs. The study was performed in healthy, metabolically stable animals to avoid interference by septic or trauma-related stress factors.

Both TPN regimens resulted in a significantly smaller weight gain (TPN-lipid, 29.8 +/- 4.0 g; TPN-CH, 30.3 +/- 4.4 g) compared with enterally fed reference animals (49.2 +/- 3.2 g; p = .007), with no difference in nitrogen balance between the TPN groups. Mucosal sucrase activity was significantly lower in both TPN groups (TPN-lipid, 8.8 +/- 1.0 x 10(-7) katal per gram (kat/g) of protein; CH: 11.9 +/- 1.6 x 10(-7) kat/g of protein) compared with enteral feeding (17.4 +/- 0.9 x 10(-7) kat/g of protein; ANOVA: p = .0007). Morphometric analysis of the small intestine revealed no differences between the two TPN groups although a significantly depressed villus height in the TPN-lipid group could be observed in comparison to enterally fed reference rats (TPN-lipid, 0.47 +/- 0.02; TPN-CH, 0.50 +/- 0.01; enteral, 0.56 +/- 0.02 mm; ANOVA: p = .0298). Light and electron microscopy revealed a normal surface architecture in all three groups of rats. Cellular immune reactivity was evaluated using a novel specific immunization protocol: animals were immunized against OVA 4 weeks before TPN. OVA-induced lymphoproliferative responses and phenotypic data from draining popliteal and mesenteric lymph nodes were evaluated after the different regimens. Results did not differ among the three groups.

In healthy rodents, short-term lipid-based and carbohydrate-based TPN regimens lead to limited mucosal atrophy with preserved surface architecture compared with enteral feeding. However, peripheral and mesenteric cellular immune responsiveness after both TPN regimens remained comparable to enterally fed reference animals. Therefore, mesenteric and systemic cellular immune reactivity does not appear to be impaired by lipid-based or carbohydrate-based TPN [181].

To detect possible interactions between lipid-based total parenteral nutrition (TPN) substrates and mononuclear phagocytes, ultrastructural in vitro and in vivo studies were carried out on material from pigs. Mononuclear phagocytes isolated from peripheral blood, phagocytosed lipid after incubation with 1 mg/ml Intralipid for 24 h. Similarly, lipid was taken up by intravascular macrophages in the lungs and liver after central venous administration of TPN containing 2.3 g/kg body weight/day of Intralipid for 5-7 weeks. Lipid accumulation was almost exclusively found intravascularly in the lungs and liver, and not in macrophages obtained from bronchoalveolar lavage fluid. A morphometric study of the lung capillaries showed that the macrophages in TPN animals had increased in size and number, and occupied a larger portion of the capillary lumina. The macrophages appeared to be activated, but the endothelial lining was well preserved. Free intravascular lipid droplets had a diameter both in vitro and in vivo of about 0.5 micron, indicating good stability of the emulsion. We suggest that the lipid uptake stimulates the macrophages and thereby plays a role in the lung tissue inflammation seen in response to long-term lipid-based TPN in pigs [182].

Intravenous lipid emulsions depress lymphocyte proliferative responses and granulocyte function at concentrations found in the blood circulation during their administration. The effects of Intralipid, a widely used intravenous lipid emulsion, were measured on immunoglobulin production in vitro by pokeweed mitogen-activated lymphocytes as a test of B-cell function. Intralipid decreased IgG, IgM, and IgA production at soybean oil triglyceride concentrations of 2.5-20 mg/ml occurring in the blood circulation during Intralipid infusion. The effects on IgM and IgA production were highest and that on IgG production lowest. Hydrocortisone-sensitive and concanavalin A-inducible suppressor cells were more sensitive to Intralipid than other cell populations. In vivo Ig production may not be equally disturbed, inasmuch as Intralipid concentrations in the lymph nodes and the spleen may be lower than in the blood circulation. However, care should be taken to prevent Intralipid concentrations from becoming high enough to depress immune responses [183].

We studied the effect of Intralipid (IL) in monocyte cultures based on the ability of the cultures to phagocytose and kill Candida albicans and produce the oxidative burst. The IL was taken up by monocytes in cultures, and these cells phagocytosed more Candida organisms than did the control cells [85 +/- 2.2% in the IL treated (1%) compared to 68 +/- 2.3% after 1 h in the control]. The percentage of killing of Candida albicans, which had been taken up by the IL-treated monocytes measured after 2 h in culture (48.3 +/- 6.0%), was no different when compared to control (47.0 +/- 5.8%). Following ingestion of IL, there was an increase in basal H2O2 production, however, the presence of the IL in the cells had no effect on the expected increase in H2O2 production following stimulation with either phorbol myristate acetate (PMA) or zymosan particles. Compared to untreated cells, a significant increase in the number of monocytes with positive nitroblue tetrazolium staining was observed in monocytes that had ingested IL (when they were stimulated with either PMA or Candida microorganisms). Similar results were obtained in monocyte-derived macrophages (i.e., monocytes in monolayer cultures for 10 days). These findings suggest that the essential monocyte functions of phagocytosis, microbicidal activity, and ability to elicit an oxidative burst are not directly altered by the conventional use of IL in clinical practice [184].

In many surgical departments it has been common practice to give patients with weight loss pre-operative parenteral nutrition before major surgery. The purpose of the present study was to elucidate the value of intravenous pre-operative nutrition in relation to the immune system. The study comprised 10 patients undergoing total gastrectomy. All patients had a weight loss of 15% of body weight or more within 6 months or 10% within 3 months. Before operation they all received parenteral nutrition for 1 week. They all had 1.5 g of protein per kg per day and energy corresponding to the basal metabolic rate + 50% as Vamin, Intralipid, and carbohydrate solutions. Before and after this treatment blood samples were taken to estimate neutrophil function (the rate of oxygen consumption and superoxide liberation, phagocytosis and intracellular lysis of Candida albicans, the concentration and consumption rate of ATP during phagocytosis, and chemotaxis) and immune globulins (IgG, IgM, & IgA). Cellular immunity (CMI) was estimated by intradermal application of seven different antigens. We found a significant increase in response to the intradermal antigens ($p < 0.01$) but no difference in any of the parameters expressing leukocyte function or immune globulins [185].

Eight healthy subjects were given Intralipid, a soybean oil emulsion, 20% intravenously for 2 h. During the infusion a significant increase in the nitroblue tetrazolium-reduction of blood monocytes was noted. Preincubation of monocytes in vitro with Intralipid (20 to 100 mg/ml) for 30 min was found to increase the ability of the cells to migrate chemotactically and to phagocytize yeast particles. On the contrary, when neutrophilic granulocytes were preincubated with Intralipid in the same concentrations for 30 min. their nitroblue-tetrazolium-reduction, chemotactic and spontaneous locomotion, as well as their ingestion of yeast particles was depressed [186].

Platinum (Pt) drugs are the most potent and commonly used anti-cancer chemotherapeutics. Nanoformulation of Pt drugs has the potential to improve the delivery to tumors and reduce toxic side effects. A major challenge for translating nanodrugs to clinical settings is their rapid clearance by the reticuloendothelial system (RES), hence increasing toxicities on off-target organs and reducing efficacy. We are reporting that an FDA approved parenteral nutrition source, Intralipid 20%, can help this problem. A dichloro (1, 2-diaminocyclohexane) platinum (II)-loaded and hyaluronic acid polymer-coated nanoparticle (DACHPt/HANP) is used in this study. A single dose of Intralipid (2 g/kg, clinical dosage) is administrated [intravenously (i. v.), clinical route] one hour before i.v. injection of DACHPt/HANP. This treatment can significantly reduce the toxicities of DACHPt/HANP in liver, spleen, and, interestingly, kidney. Intralipid can decrease Pt accumulation in the liver, spleen, and kidney by 20.4%, 42.5%, and 31.2% at 24-hr post nanodrug administration, respectively. The bioavailability of DACHPt/HANP increases by 18.7% and 9.4% during the first 5 and 24 hr, respectively [187].

14. CONCLUSIONS

Henry Sigerist said, more than 50 years ago: *"I personally have the feeling that the problem of cancer is not merely a biological and laboratory problem, but it belongs to a certain extent to the realm of philosophy... All experiments require certain philosophical preparation. And I have the*

feeling that in the case of cancer many experiments were undertaken without the necessary philosophical background, and therefore proved useless" [96].

Urotherapy is suggested as a kind of immunotherapy for cancer patients. Unlike the clonal immunotherapy the urine of the cancer patients contain the many tumor antigens which constitute the tumor. Oral auto-urotherapy will provide the intestinal lymphatic system the tumor antigens against which they may produce antibodies due to non-self-recognition. These antibodies may be transpierced through the blood stream and attack the tumor and its cells [174].

Intralipid can increase the response to the cancer antigens in the intestinal lymphatic system against which antibodies may be produced.

The use of oral intralipid together with auto urotherapy in patients with cancer is first suggested in the medical literature. Intralipid can increase the response to the cancer antigens in the intestinal lymphatic system against which antibodies may be produced. These antibodies may be transpierced through the blood stream and attack the tumor and its cells.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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