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Review

Serial Immune Function Testing to Predict Clinical Disease Relapse in Patients with Solid Tumors

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Introduction

The assessment of immunity in humans and the role that such assessments play in influencing choices in cancer patient management have been the subject of several excellent reviews [20, 23, 26, 53]. Single-point immune assessments of cancer patients have provided useful information about the relationship between stage of disease, patient prognosis, and the functional integrity of patient immune reactivity. A full definition of those immunological changes associated with malignant disease, however, requires investigations dealing with the serial immune monitoring of cancer patients. This type of investigation would be better suited to describe the sequence of immune changes which occur when a patient's disease course moves from a state of microscopic nondetectable disease to a state of clinically overt, macroscopic disease. Such a situation would follow the local surgical excision of a primary tumor where in time residual local microscopic disease or metastatic deposits of microscopic tumor have grown to present with clinically overt recurrent cancer. Specific clinical circumstances where this occurs would include surgically resected Stage I and II lung cancer, Dukes C bowel cancer, Stage I (deep penetration) and Stage II malignant melanoma, and Stage II breast cancer. Following tumor resection in these stages of disease patient immune function is generally normal or has been restored to normal. At the time of tumor recurrence immune function will be found to be variably but grossly compromised. What remains to be established is the sequence and the exact nature of the changes in immune function that occur prior to the observation of gross immunological impairment associated with newly detected advanced-stage recurrent disease. That type of information might make it possible to intervene with an immunotherapeutic manipulation to prevent or to modify the immune changes that occur. That in turn might delay, prevent, or reduce the size and degree of spread of recurrent disease.

The intent of this approach to immune assessment of cancer patients would be to identify a series of suitable immunological parameters to monitor and to determine when changes in those parameters might indicate clinical tumor recurrence and patient relapse. That could offer the possibility of timely therapeutic interventions to reverse or to prevent undesirable immune changes and so perhaps affect the clinical course of monitored patients. This review will focus on that problem.

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The purpose of the review is to consider some of the information which has been obtained in sequential studies of cell-mediated immune function in cancer patient populations either receiving various forms of therapy or being monitored following primary surgery with no specific treatment. The review will be restricted to the nonlymphoreticular solid tumor malignancies, since immune function abnormalities seen with tumors of the immune system present unique problems of interpretation. Alterations in humoral immunity, and particularly in serum 'blocking' factors, will not be dealt with in depth in this review since that subject has been reviewed elsewhere [50].

Serial Immunological Assessment of General Immunocompetence in Patients with Solid Tumors

Serial immunologic testing of patients with solid tumors has relied chiefly on assays of general immunocompetence; these determinations include quantitation of leukocytes and leukocyte subpopulations, assessment of lymphoproliferative responsiveness to antigens and to mitogens, and assessment of delayed cutaneous hypersensitivity (DCH) to dinitrochlorobenzene (DNCB) and recall antigens. Some, but not all, single-point studies have demonstrated a relationship between lymphocyte numbers, lymphocyte subset proportions, stage of disease, and patient prognosis. In those studies there was a trend toward decreasing lymphocyte values with increasing tumor burden [26, 53]. Also, within each stage of disease, patients with normal lymphocyte values appeared to fare better than those with depressed values [26, 53]. It is not surprising then that serial determinations of lymphocyte values have demonstrated, in some situations, an association between decreasing lymphocyte levels and disease relapse.

In patients with lung cancer, a fall in the absolute lymphocyte count, a decrease in the percentage of T cells, and a decline in the proportion of active T cells have each been associated with disease relapse. Anthony et al. studied, at monthly intervals, 30 patients with lung cancer treated by surgery, radiotherapy, BCG, or combinations thereof [1]. Immunotherapy led to increases in T cell percentages in about half the cases studied. A fall in the absolute lymphocyte numbers and T-cell percentages preceded disease relapse and clinical deterioration by 2–4 months. This occurred in approximately half the 14 patients who relapsed, and was associated with a relative increase in B cells measured by EAC rosettes.

In the other patients no consistent changes in these parameters were noted. This pattern was not dependent on previous therapy. In the study of lung cancer patients by Dellon et al. there was an inverse relationship between T-cell levels and stage of disease prior to surgery in patients with small cell, squamous, or large cell tumors of the lung [13]. In patients with adenocarcinoma, T cells were reduced to the same extent in patients with localized or disseminated disease. In subsequent serial studies, a decline in T-cell levels preceded the clinical detection of recurrent disease by 2–3 months. Patients who showed stable or rising T-cell values following definitive surgical resection did not suffer disease relapse. However, patients with squamous cell carcinoma who remained free of disease had persistent low levels of T cells.

The decline in T-cell percentages reported in serial studies of lung cancer patients has been attributed in some studies to a decline in that portion of the total T-cell population known as the active T cells. Active T-cell percentages have been found to decline in lung cancer patients prior to disease relapse. In the study by Oldham et al. [42] a pattern of dereasing active T-cell percentages without a concomitant decline in total T cells was seen in lung cancer patients prior to or coincident with clinical detection of metastasis. Patients with resectable disease received surgery ± BCG, patients with nonresectable localized disease received partial resection, radiation, chemotherapy with cytoxan, methotrexate, and vincristine ± BCG, and patients with disseminated disease received partial resection, radiation therapy to metastatic disease, chemotherapy with cytoxan, methotrexate, and vincristine ± BCG. On average, active T cells declined to subnormal levels 3 months prior to confirmation of disease relapse or disease progression. This was also seen in those patients whose active T-cell percentages persisted at subnormal levels throughout the study. However, each of these patterns was also seen in patients who remained free of disease. In contrast, patients who showed normal active T-cell levels throughout the 2-year follow-up period or who demonstrated an increase in active T cells from subnormal to normal levels during the study remained free of disease.

This same general pattern of decreasing lymphocyte, T cell, or active T-cell level in individual patients coincident with clinical detection of disease relapse has been found in other tumor systems. Wybran and Fudenberg, who first used the active T-cell rosette assay to study cancer patients, showed that active T-cell percentages generally followed the clinical course of individual patients [59]. Active T cells declined prior to clinical relapse in a patient with osteogenic sarcoma treated with radiation therapy + transfer factor or during progressive clinical deterioration in a patient with ovarian cancer; improved coincident with clinical response to chemotherapy with prednisone + triiodothyroxine in a patient with disseminated breast cancer; and remained stable in patients with breast or colon cancer during periods of stable disease. These results were extended by this same group in their subsequent studies [34]. In those studies, active T cells declined several weeks prior to detection of recurrent disease in patients with surgically resected osteogenic sarcomas who had received radiation therapy with or without chemotherapy at a time when total T cells were normal. Total T cells were not found to decrease even under the influences of therapy until the terminal phase of progressive disease. Analogous findings have been reported by several other groups in a variety of solid tumor settings, including gastric carcinoma and head and neck cancers [31, 48, 57, 60], although in some of those studies depression of active T-cell numbers without changes in active T-cell percentages has been the indicator of clinical disease recurrence.

The clinical utility of serial immunological monitoring of cancer patients is illustrated by the study of Brooks et al. in patients with malignant brain tumors [7]. In that study, 82 patients with primary malignant brain tumors who had undergone subtotal resection of disease and 5,000 rads whole-brain irradiation were assessed with a battery of tests preoperatively and every 1–2 months thereafter until clinical reactivation of disease. On average, absolute lymphocyte counts and T-cell percentages declined and nonspecific serum blocking increased 4–9 weeks before clinical evidence of recurrent tumor could be appreciated. Indeed, no evidence of recurrent tumor could be found at the time of a significant alteration in these immune parameters. There was no significant correlation between preoperative immunological parameters and subsequent clinical course in these patients. The inability to predict stage of disease, responsiveness to therapy, and subsequent clinical course from preoperative determinations of leukocyte values has, for the most part, also been the rule for patients with breast cancer, melanoma, and sarcomas; there has been less consistency in studies of patients with colon carcinoma [39, 52, 53, 54].

Clearly, all studies do not agree and a consensus concerning the usefulness of leukocyte quantitation in cancer patients has been difficult to achieve. This dilemma can be appreciated by considering several examples. Wybran reported a decline in active T cells without a change in total T cells prior to clinically detectable disease progression in patients with surgically resected melanoma [59]. But Ninger et al., in follow-up studies of patients with surgically resected melanoma, have observed just the opposite in some patients, no change in other patients, and similar changes in still other patients immediately prior to or coincident with clinical recurrence of melanoma [40]. No consistent pattern of change in total or active T cells prior to clinical relapse was found in some patients with lung cancer who received radiation therapy, BCG, or both [30]. In this last study, total T-cell percentages were decreased in most, but not all, patients who suffered disease relapse; changes in active T-cell percentages were less consistent in this group and showed an equal tendency to decrease, increase, or remain the same. It is of interest to note that investigators who had originally reported an association between clinical disease relapse and decreased active T-cell percentages have been unable to confirm their original finding in their later studies [43].

Studies of this nature have generally provided interesting, but inconsistent, information. This is due primarily to the small numbers of patients available for serial assessment and the infrequency of monitoring. Some investigations also suggest that frequent test intervals are necessary to establish the sensitivity and reproducibility of this approach [30].

Three other studies of serial leukocyte determinations in cancer patients deserve mention. Leukocyte populations other than the lymphocyte have been monitored for indication of disease relapse or disease progression in serial immunological studies. In that regard, Hedley et al. reported that monocyte maturation to macrophages in vitro and nitroblue tetrazolium dye (NBT) reduction by peripheral blood monocytes were diminished prior to or coincident with clinically detectable recurrence of melanoma [24]. Also, the value of serial leukocyte determinations need not be limited to post-surgical settings. McMahon et al. demonstrated that a persistent lymphocytopenia of at least 3 week's duration prior to surgery

could distinguish bronchogenic carcinoma patients from those with benign lesions with 95% accuracy (based on 40 patients) [38]. Finally, the development of monoclonal antibodies capable of distinguishing an ever-expanding number of leukocyte subsets should allow for more precise serial leukocyte determinations in cancer patient populations. It will be important to perform these studies with a variety of available reagents to determine which of these reagents will be clinically useful. Using commercially available reagents reported to measure helper/inducer cells and suppressor/cytotoxic cells it has been reported that patients with disseminated solid tumors do not exhibit significant alterations in T-cell subset distributions, and therefore serial monitoring of these subsets with that set of reagents would not be expected to be a useful procedure to predict disease recurrence [22]. This approach was, in fact, not useful in the study by Karavodin et al. [29]. In that study, sequential determinations of total T cells, helper/inducer T cells, and suppressor/cytotoxic T cells in the peripheral bloood of melanoma patients with commercially available monoclonal antibody reagents did not show fluctuations which could be correlated with clinical evidence of tumor progression.

The other in vitro technique for assessing general immunocompetence in humans that has been most frequently employed for serial studies of cellular immunity in solid tumor patients is the lymphoproliferation assay. Most studies have used T-cell mitogens or allogeneic lymphocytes to induce lymphocyte DNA synthesis in peripheral blood mononuclear cells. These assays show wide biological variation even when performed sequentially with cells from normal healthy subjects. Such normal variation must be considered in analysis of the results of single-point studies in cancer patient populations. For example, in one study of patients with bladder cancer [9] the lymphoproliferation responses of PBMC from individual subjects in the normal control group varied by as much as 70% over a period of several weeks. Another problem which must be appreciated is that antineoplastic therapies produce variable effects on the lymphoproliferation assay. The responsiveness of PBMC from nasopharyngeal cancer patients to PHA was found to recover later and to a lesser extent than the responsiveness to ConA following radiation therapy [33]. Depressed PHA responses in cancer patients have also been found to improve following surgery [41] and combination chemotherapy [5]. Cancer patients brought into complete and permanent remission of disease with therapy may remain immunodepressed for years thereafter [15].

The relationships already discussed concerning disease relapse and fluctuation of leukocyte values are often paralleled by similar fluctuations in lymphoproliferative responsiveness. Oldham et al. [42] showed a good correlation between declining values for percentages of active T cells, PHA responses, and disease relapse in their lung cancer patients serially monitored following surgical resection. As was the case for active T cells in that study, PHA response changes preceded clinical detection of recurrence. Somewhat similar findings were reported by Gross and Eddie-Quartey in their studies of surgically resected lung cancer patients who received BCG [19]. In that study, lymphoproliferative responses to PHA and to PPD declined significantly 3 months prior to clinical detection of recurrence. This was not the case for resected melanoma patients. The manner in which the lymphoproliferative data was expressed was important since significant declines were found only when responsiveness was expressed as an 'optimal adjusted response' which takes into account the lymphoproliferative reactivity produced by suboptimal stimulating concentrations of antigen or mitogen. Depressed PHA responses in lung cancer patients in association with increases in monocyte suppressor cell activity and clinical disease relapse have also been observed in a study where surgically resected lung cancer patients were randomized to receive either no therapy, 3-monthly injections of Freund's Complete Adjuvant, or 3-monthly injections of Freund's Complete Adjuvant emulsified with extracted lung tumor-associated antigens [6]. These findings suggest that the depressed PHA responses seen in other immune monitoring studies of cancer patients may also have resulted from abnormal immunoregulation mediated by suppressor monocytes. In that same study, T cell and active T-cell changes could not be correlated with clinical disease relapse.

A different relationship was reported for lymphocytes and lymphoproliferative responses in patients who relapsed with breast cancer [17]. In the study by Glas et al. [17] lymphocyte counts and lymphoproliferative responses were compared among 'recurrent' and disease-free patients. In the patients who developed recurrent disease, lymphocyte counts were depressed at the time of clinical evidence of metastasis compared with pretherapy values. This was not the case in patients who remained free of disease. Lymphoproliferative reponses to PPD were depressed in patients with recurrent disease compared with the values seen in patients who were free of disease; on an individual basis they were not significantly different from pretherapy values. PHA reactivity in the recurrent patients was unchanged when pretherapy and disease recurrence assessment points were compared; in the disease-free patients, a significant increase in reactivity was found. However, since these conclusions were based on the results of single-point studies performed following surgery and at the time of disease relapse only, the temporal relationship between changes in immune function and changes in disease status could not be appreciated.

Payne et al. [45] have monitored lymphoproliferative responses in patients with colon cancer following surgical resection of primary disease. Of 10 patients who relapsed during the study, nine demonstrated depression of PHA-induced lymphoproliferation preceding or coincident with clinical detection of disease recurrence. Five of those patients had diminished PHA responses at least 3 months before their CEA levels became abnormal. Some fluctuations in PHA response were also seen, however, in some patients who did not suffer disease relapse. Golub et al. monitored melanoma patients for lymphoproliferative responsiveness to mitogens (PHA, PWM, and ConA) and antigens (PPD and allogeneic cells) during therapy with tumor cell vaccines [18]. They found that mitogen responses were stable during therapy, PPD responses increased during therapy in those patients who were originally tuberculin negative, and MLC responses increased for all patients during therapy. In the six patients who eventually relapsed, only the MLC response diminished prior to clinical detection of recurrence. In surgically resected melanoma patients, consistent normal levels of DNA synthesis in response to stimulation by mitogens and PPD have been found throughout periods of disease relapse and disease progression [20, 51]. Cunningham et al. serially monitored breast cancer patients receiving adjuvant chemoimmunotherapy with the PHA assay [11]. Patients received either phenylalanine mustard or 5-FU + cytoxan or 5-FU + cytoxan + BCG at regular intervals for up to 1 year following mastectomy. Following surgical resection of tumor there was some recovery

of PHA responsiveness. Adjuvant therapies did not consistently influence PHA responses in any of the three groups. No consistent pattern of PHA responsiveness was seen in association with disease recurrence in the 39 patients who relapsed.

Some of the difficulties in applying and interpreting lymphoproliferation assays for serial studies of cancer patients have been considered by Dean et al. [12]. Lymphoproliferative responses to PHA and to alloantigens in the MLC were tested in a large group of surgically resected lung cancer patients treated with BCG. The manner in which the data were expressed had a significant impact on the interpretation of the results. When lymphoproliferative responses were expressed as stimulation indices, the variation seen with normal subjects was large and the values obtained for cancer patients often appeared comparable to those of normal subjects in longitudinal tests. When the raw data were expressed as either net counts per minute (ncpm) or as a relative proliferation index (defined as ncpm patient divided by mean ncpm of three normal subjects tested on the same day as the patient), the da-to-day variation in activity was substantially reduced and the patient group demonstrated an inverse relationship between immune reactivity and disease progression.

Thus, the difficulties in analyzing the results of serial studies of leukocytes in cancer patients also apply to the lymphoproliferation assay. The results obtained in applying these techniques show great variability. Investigators applying them do not always report their results in a uniform way, making comparisons between studies difficult.

Delayed cutaneous hypersensitivity responses (DCH) to the primary skin sensitizing agent DNCB and to recall antigens have been widely used to test the immunocompetence of solid tumor patients. The response to DNCB is considered one of the most sensitive indicators of a change in the tumor status of a patient. The response to DNCB depends on the immunocompetence of the afferent, central, and efferent portions of the immune reaction mechanism. Positive responses to DNCB have been used to predict clinical responses to chemotherapy in breast cancer [10, 55]. DNCB responses have been related to disease-free interval and survival in various stages of bladder cancer [8]. Comparative studies suggest that different types of malignancy show different patterns of change with advancing disease in DCH tests [4]. Colon cancer patients and head and neck cancer patients appear to lose DCH reactivity even in early stages of the disease, while patients with gastric cancer, lung cancer or breast cancer often maintain reactivity in later stages [4, 53].

In one study of patients with melanoma or soft tissue sarcoma (Stages I, II, or III), patients tho were not reactive to DNCB or who converted from positive reactivity to negative reactivity had an 80% probability of suffering disease relapse [14]. The maintenance of positive reactivity or conversion from negative to positive reactivity was associated with tumor control. Skin test reactions to recall antigens were also serially tested in this study, but did not correlate with clinical course in either melanoma or sarcoma patients. Most patients who suffered disease relapse maintained positive DCH to at least one recall antigen. The maintenance of vigorous reactivity to recall antigens throughout the course of disease relapse and tumor progression has also been reported for breast cancer [56]. Similarly, patients with lung cancer whose active T cell and PHA responses declined in association with disease relapse continued to respond to recall antigens in DCH tests [42]. Neither DNCB responsiveness nor recall antigen responsiveness has been found to correlate with reactivation of disease in glioma patients [7]. The majority of patients with disseminated breast cancer who maintained or increased their positive responsiveness to DNCB during a 1-year follow-up period remained free of disease. During that time more than one-half of those patients whose DNCB responses declined suffered recurrent disease. The improved survival group with good DNCB reactivity consisted largely of patients with osseous or visceral disease [10].

An association between diminution of DNCB reactivity and disease relapse has also been reported for patients with surgically resected bladder cancer and renal cancer [36]. In the same study, there was no such correlation for patients with prostatic cancer. In another study of patients with bladder cancer [8], clinical course was related to DNCB reactivity for patients with superficial disease but not for patients with invasive or metastatic disease. There was significantly greater variability in skin test responsiveness in the cancer patient population for all stages of disease than there was in the healthy control population.

Serial Assessment of Tumor-Associated Immunity in Patients with Solid Tumors

Immune responses to antigens associated with tumor cell surfaces by host T cells, B cells, macrophages, K cells, and NK cells have been described in most cancer patient populations. Serial assessments of immune responses against neoplastic tissues have been conducted in an attempt to correlate fluctuations in patient anti-tumor immunity with alterations in patient tumor status. These studies have relied largely on the DCH test using tumor cells or tumor cell extracts in vivo and on assays of lymphoproliferation, leukocyte migration inhibition (LMI), and leukocyte adherence inhibition (LAI) in the presence of extracts of neoplastic tumor tissues or on assays of cytotoxicity against allogeneic or autochthonous target cells. It is not usually clear whether the activity being measured is directed wholly, in part, or not at all against determinants found on the patient's own tumor cells. Also, the question of specificity of these reactions for neoplastic vs normal tissues has yet to be resolved, and this remains an exceedingly controversial area in tumor immunology. Nevertheless, the limited number of serial studies that have been conducted have provided some interesting biological information.

In many serial studies of this sort in patients with solid tumors, no correlation has been demonstrated between fluctuations in anti-tumor immunity and disease progression. McCoy et al. [37] used the LMI assay to monitor the responses of surgically resected lung cancer patients to allogeneic tumor cell extracts. The results suggested that most lung cancer patients, regardless of stage of disease, can respond to lung tumor-associated antigens. Patients tested at clinical relapse and even patients tested within 1 month of death were found to react to tumor antigen extracts in the LMI assay.

Serial studies of prostatic cancer patients with the LMI and LAI assays using tumor extracts demonstrated some degree of cancer-related immunity in these assays. Reactivity was detected in the majority of patients regardless of stage of disease or treatment history. Patients with benign prostatic hypertrophy did not demonstrate such reactivity [16]. In patients with gliomas there was no relationship between reactivation of disease and cytotoxicity against glioma target cells [58].

In one study of patients with Stage I or Stage II melanoma who received adjuvant chemotherapy with DTIC [2] serial determinations of anti-tumor cytotoxicity could not predict disease recurrence in patients who relapsed and indeed, did not fluctuate in patients during progressive clinical deterioration. In another study of surgically resected melanoma patients [3] treatment with chemotherapy (methylCCNU) and immunotherapy (BCG + allogeneic melanoma cells) led to a significant increase in anti-melanoma cytotoxicity; but that event was not associated with tumor regression or with improved patient survival.

A high percentage of those patients with Stage I or Stage II melanoma who were serially monitored postoperatively and who demonstrated persistent anti-tumor cytotoxicity against established melanoma cell lines were found to relapse within 1 year [32]. In the same study, those patients who demonstrated a progressive decline in anti-tumor cytotoxicity following surgery remained disease-free. This is in contrast to earlier reports, which suggested that melanoma patients with at most minimum residual tumor burdens have higher levels of anti-melanoma cytotoxicity than those patients with greater tumor burdens [25]. Blocking of anti-tumor cytotoxicity by patients' serum coincident with disease recurrence was also reported in those studies [25].

Another predominant pattern of anti-tumor immune reactivity which has been observed in serial studies of solid tumor patients is an initial decline in anti-tumor reactivity following surgical resection or cytotoxic therapy, followed by the reappearance of anti-tumor reactivity coincident with disease recurrence. Early studies by O'Toole [44] demonstrated that bladder cancer patients generally have detectable anti-tumor cytotoxicity prior to surgery. Following surgery, most patients lose reactivity. Those patients who relapse do not demonstrate reactivity for extended periods of time (up to 1 year), but then regain reactivity at or about the time of clinical detection of recurrent disease. Patients who achieve tumor control lose reactivity following therapy as well, but rapidly regain and maintain that reactivity. Similar results have been obtained in serial studies of patients with breast cancer by Rieche et al., who monitored breast cancer patients with the LMI assay [46]. They observed positive reactivity prior to mastectomy, a decline in reactivity within weeks following surgery, and the reappearance of reactivity immediately prior to clinical detection of metastatic disease. These results have been confirmed in a separate series of breast cancer patients using the LAI assay [35]. In that study, most patients lost reactivity to tumor antigen preparations following mastectomy, even in the presence of residual disease. Those patients who regained LAI responses to breast tumor extracts were found to have recurrent disease within 4 months following the reappearance of anti-tumor reactivity.

The other major pattern of anti-tumor immune reactivity that has been found in serial studies of solid tumor patients is a loss of reactivity prior to or coincident with disease relapse. That has been the case in some studies of surgically resected Stage I or Stage II breast cancer patients, some of whom received adjuvant radiation therapy and were serially monitored using the LMI assay with allogeneic tumor tissue extracts [27, 28]. In the study by McCoy et al. [37], some association between declining LMI reactivity to 3 *M* KCl extracts of a breast cancer cell line and disease recurrence was found. LMI reactivity tended to decline from 1–6 months prior to clinical relapse. Following disease relapse, low levels of LMI reactivity persisted. In one study of patients with bladder cancer, anti-tumor cytotoxicity was low prior to surgery, improved following tumor resection, and diminished again with recurrence [21]. The decline in anti-bladder tumor cytotoxicity seen with disease relapse was not due to a generalized suppression of cytotoxicity, since nonspecific cytotoxic activity was normal during this time. In a study of patients with melanoma who were treated by regional BCG therapy [47], 70% of those patients who exhibited disease progression demonstrated a progressive loss of anti-melanoma cytotoxic activity in vitro. The remaining patients in the progressive disease group maintained anti-tumor cytotoxicity. Those patients with stable disease who were observed during the same period demonstrated either stable positive cytotoxicity or progressive increases in cytotoxicity. The maintenance of positive DCH reactivity to lung cancer antigens in patients who were vaccinated with these materials emulsified in Freund's Complete Adjuvant has been associated with tumor control [49].

Concluding Remarks

There are major problems in conducting serial immunologic assessments of cancer patients, which need to be addressed in the near future. First, there is the problem of determining which assays are useful to perform. In that regard, it would be necessary to determine which immune responses are affected by a particular malignancy and how those effects vary with the clinical course of the disease. As can be appreciated from this review, even today there is no clear consensus in the literature on the usefulness of monitoring a single cell-mediated immune function in a specific malignant disease situation. Some of the more recent general cell-mediated immune assays which test functions such as natural killing, antibody-dependent cellular cytotoxicity, spontaneous macrophage-mediated cytotoxicity, and elements of the immunoregulatory cell network might be considered in this regard. Immunoregulatory cell changes might precede and lead to the depression of immune effector function. Should that happen then clinical tumor recurrence in surgically resected cancer patients who are left with at most minimal residual microscopic disease may be a direct result of the failure to control abnormal immunoregulation in those cancer patients. Alternatively, regrowth of residual tumor might lead to the induction of abnormal immunoregulation, resulting in eventual depression of those immune effector functions responsible for controling tumor cell growth. An appreciation of this abnormality suggests the means of therapeutic intervention with agents capable of selectively inhibiting monocyte or lymphocyte suppressor cell mechanisms. These could be used alone or in combination with cytotoxic drugs in the adjuvant therapy of surgically resected cancer patients.

Measurements of tumor-associated reactivity which test antigen recognition, proliferation, differentiation, and eventual tumor cell attack capabilities most likely should concentrate on using the patient's own tumor cells. The results obtained with these kinds of assessments would need to be considered in light of both the heterogeneity of the patient's tumor and their general immune function status, since immune-mediated tumor cell destruction in vivo is thought to depend on both tumor antigen-reactive cells and the activation of nonspecific effector cell mechanisms.

A major obstacle which needs to be overcome is the problem of standardization of assays. This task is particularly difficult for assays requiring living immunological cells since

(i) cell-mediated immune assays are semi-quantitative at best; (ii) they are subject to extreme biological variability; and (iii) they are generally performed with heterogeneous cell populations and crude or at best semi-purified antigen or mitogen preparations. Here, the trend towards using cryopreserved normal responder cells as controls should be encouraged; perhaps these tests might also include the use of cryopreserved autologous patient cells as well where that is possible. Also, the variation inherent in any immune function assay used for serial monitoring of cancer patients should be quantitated in each laboratory to permit a more rational basis on which to analyze the data obtained in serial tests of cancer patients.

Finally, there is a need to establish how to apply immune function tests and how to analyze and express the data obtained in a cogent and clinically useful manner. Here, patient compliance issues come into play; for example, it is not clear how frequently certain tests should be performed. Also, one must have a means of factoring in the effects of age and treatment history on the results of immune function assays if these tests are to be applied to specific individuals. The ability to test individual patients in specific immune function assays and to obtain data meaningful for other patients who would be tested at other institutions is another obstacle which has not been adequately addressed, although attempts at arriving at uniform means of expressing data have been made.

Clearly, there are significant obstacles to overcome before serial immune testing can be applied to individual cancer patients in a clinically meaningful fashion. Still, the effort should be made. This is based on the fact that serial immune testing of cancer patients could lead to significant information about the biology of the host-tumor relationship which potentially could be applied to immunotherapeutic intervention in this disease. Serial tests could identify the sequence of immune function changes which accompany clinical disease relapse. Those changes might reflect the nature of the events responsible for disease recurrence. It is still not clear whether the development of immune depression in patients at risk for disease recurrence is responsible for or is a consequence of growth in tumor burden. It could be that immune depression permits exacerbation of tumor through mechanisms which are thought to contribute to primary onconeogenesis. In contrast, reactivation of quiescent tumor might lead to immune depression prior to clinically obvious disease relapse as a secondary phenomenon. More detailed study of the immunoregulatory abnormalities associated with cancer is required. An appreciation of the defects found in patients prior to periods of clinical disease relapse might also suggest means of external immunological intervention with specific vaccines, general biological response modifiers, cloned immune effector moieties or a combination of these approaches. This might block or subvert the heretofore inexorable processes responsible for clinical disease relapse in patients who, for a time, have stable disease but then succumb with reactivation of tumor.

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