Clin. exp. Immunol. (1971), **9**, 713–719.

DELAYED CUTANEOUS SENSITIVITY REACTIONS TO AUTOLOGOUS BURKITT LYMPHOMA PROTEIN EXTRACTS

RESULTS OF A PROSPECTIVE TWO AND A HALF YEAR STUDY

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(*Received* 4 *May* 1971)

SUMMARY

Positive delayed cutaneous sensitivity reactions to protein extracts of autologous Burkitt lymphoma cells were observed in fifteen of thirty patients tested in clinical systemic remission and in only one of sixteen patients tested with active extradural disease. Positive responding patients who eventually relapsed had significantly longer remission durations than did their negative responding counterparts. Seven patients with positive reactions were negative when retested in relapse. Positive reactions were not observed at extract protein concentrations of 0·1 mg/ml and were maximally evident at 1 mg/ml. Negative skin tests could not be attributed to generalized anergy because of the simultaneous observation of positive reactions to common antigens in over half the negative responders. Cutaneous reactivity to autologous tumour extract may serve as a sensitive test for extradural disease activity, and, because of its association with remission duration, suggests a role for the specifically sensitized lymphocyte in tumour regression and prognosis.

INTRODUCTION

A clinical study of cutaneous delayed sensitivity reactions to extracts of autologous Burkitt lymphoma cells has been previously reported from this Centre (Fass, Herberman & Ziegler, 1970). In that study positive reactions, seen in only one of twelve patients with active disease and in seven of twelve patients in clinical remission, were found to correlate with prolonged remissions. A total of thirty patients with Burkitt's lymphoma have now been tested over the last 2½ years yielding several additional observations. This paper is a report of our continuing experience with this test.

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MATERIALS AND METHODS

Patient selection and management

All patients admitted to the Lymphoma Treatment Centre between August, 1968, and November, 1970 with biopsy-proven Burkitt's lymphoma were potential candidates for this study. The only patients not studied were those lacking sufficient biopsy material for tumour extract preparation and those few who died before achieving clinical remission. All patients were uniformly staged and treated according to a previously described protocol (Ziegler *et al.*, 1970).

Cell extract preparation

The method for preparing the autologous tumour and autologous peripheral lymphocyte extracts used as controls has been previously reported in detail (Fass *et al.*, 1970). Cell suspensions were prepared from freshly biopsied tumour tissue. These suspensions were frozen and thawed following which they were serially exposed to saline solutions of decreasing tonicity (from isotonic saline to distilled water). After each hypotonic lysis the suspension was centrifuged and the supernates collected and pooled. The pooled supernatant fluids were then concentrated over 36 hr by pressure dialysis at 4°C to a final volume of 1 ml. Following preparation the protein concentration of the extracts was determined by the method of Lowry *et al.* (1951); the extracts were then made up to three protein concentrations (1 mg/ml, 0·5 mg/ml and 0·1 mg/ml), divided into 0·1 ml aliquots in tuberculin syringes, and stored at −70°C until testing. All antigen preparations were cultured on blood agar prior to testing, and all were found to be bacteriologically sterile.

Administration and interpretation of skin tests

One-tenth millilitre of the following antigens were intradermally applied to the back in a vertical pattern along the axis of the sacrospinalis muscle group: all autologous tumour and control lymphocyte protein extracts, intermediate strength PPD (Tuberculin, Purified Protein Derivative, Intermediate test strength, Parke-Davis and Company, Detroit, Michigan), streptokinase-stroptodornase (Streptokinase-Streptodornase Varidase diluted 1:50 (each ml containing 400 U streptokinase and 100 U streptodornase), Lederle Laboratories, Pearl River, New York), mumps (Mumps Skin Test Antigen, Eli Lilly and Company, Indianapolis, Indiana), and candida albicans (Dermatophytin 'O' 1:100 diluted 1:20, Hollister-Stier Laboratories, Spokane, Washington). A positive skin test was defined as one having a diameter of at least 5 mm induration maximal at 48 hr. All skin tests were read by at least two individuals who had no knowledge of the specific antigen test locations. Skin punch biopsies were performed on all positive tumour extract skin tests and on the corresponding autologous lymphocyte control sites. These histological preparations were independently evaluated by three observers who did not know the clinical interpretation of the skin tests being read. Perivascular mononuclear (lymphocyte and macrophage) cell accumulation was required to establish a histological diagnosis of reactivity.

When feasible, patients were tested prior to chemotherapy. All patients were tested upon attaining complete remission and at the time of relapse.

RESULTS

All positive skin tests fit the clinical criteria for delayed sensitivity reactions in that maximum

induration of 5 mm or more was observed at approximately 48 hr. An 83% correlation was observed between clinical and histological interpretations of cutaneous reactivity. Perivascular mononuclear infiltrates were seen on histological section of all clinically positive tumour extract reactions and on five of fifteen biopsied clinically negative sites. Because of the difficulty in deciding exactly how many perivascular mononuclear cells constituted a delayed sensitivity reaction, the clinical rather than the histological interpretations were used to determine reactivity for the purpose of this study.

The protein concentration of the extract was found to be an important determinant of cutaneous reactivity. Ten of fifteen patients with positive reactions to the 1 mg/ml tumour protein extract failed to respond to the 0·5 mg/ml challenge, and no patient reacted to the 0·1 mg/ml concentration. The 1 mg/ml concentration was therefore taken as the critical antigenic challenge.

A total of thirty-seven patients were tested in remission. Seven, all of whom were positive to autologous tumour extract, reacted to the 1 mg/ml control lymphocyte extract as well and were therefore eliminated from the study. The responses to autologous tumour extract in the remaining thirty evaluable patients are summarized in Table 1.

TABLE 1. Tumour extract response correlated with disease activity

		Tumour skin test reactivity	
Disease activity	No. Tested	No. Positive	No. Negative
Active	16	1	15
Remission	30	15	15
Relapse	7	0	7

Sixteen patients were tested at the time of initial active systemic disease, and all but one were negative to autologous tumour extract. The one positive responder had a small tumour localized to the right maxilla. Of the remaining fifteen patients, four reacted to at least one of the other antigens at the time of tumour extract unresponsiveness.

Thirty evaluable patients were skin tested in remission; fifteen had positive reactions, and fifteen failed to react to the autologous tumour extracts. Four of the positive responders, although in systemic remission, had tumour cells in the cerebrospinal fluid at the time the positive skin tests were elicited. Ten of the fifteen responders (67%) and eight of the fifteen nonresponders (53%) reacted to at least one of the battery of four other antigens used.

Seven patients, all of whom were skin test positive in remission, were retested in relapse. All had lost reactivity to the tumour extract although only two lost reactivity to other previously positive antigens. In three of these seven patients skin test conversion antedated clinical relapse by 12, 23 and 30 weeks respectively. We have observed only one instance of the loss of positive reactivity not associated with clinical relapse. This one patient is still in remission 46+ weeks following skin test conversion. No patient skin test negative during an initial remission converted to positive during subsequent remissions.

Since the start of this study 2½ years ago, three of the fifteen skin test positive patients

and five of the fifteen skin test negative patients have died. The median survival in the positive group was 55 weeks (range 28–93) as compared to a median of 16 weeks (range 8–43) in the negative group. These numbers are currently too small for statistically significant evaluation.

An actuarial analysis of the remission duration of both groups is shown in Fig. 1. Seven of the nine relapsing patients in the skin test negative group relapsed in less than 10 weeks;

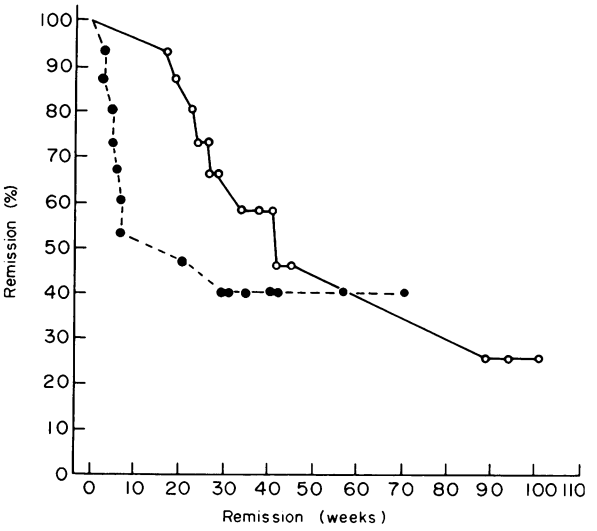

FIG. 1. Cutaneous reactivity to tumour extract related to remission duration. ○ Skin test positive (n = 15); ●, skin test negative (n = 15).

no skin test positive patient relapsed within that period of time (P = 0·005 by Wilcoxon analysis). Following the first 10 weeks, however, no difference is evident from these curves. No difference was noted between the two groups with respect age, sex, disease stage, disease site, or relapse rate.

DISCUSSION

The successful application of immunotherapy to the treatment of human cancer rests upon the discovery of antigens qualitatively unique to tumour tissue. If a host response to such an antigen can be demonstrated, and if the presence of this response is found to correlate positively with prognosis, a major advance toward the rational application of immunotherapy will have been achieved.

Circulating antibodies directed against autologous and allogeneic Burkitt lymphoma cells have been reported by several investigators (Fink *et al.*, 1969; Henle *et al.*, 1969; Klein *et al.*, 1966; Old *et al.*, 1966; Osunkoya, 1967). An attempt to correlate antibody titre with changing disease activity in a single individual was inconclusive (Klein *et al.*, 1969) however, and a controlled study failed to demonstrate any beneficial effect on the active disease of 'immune' allogeneic serum infusion (Fass *et al.*, 1970).

The present report can be best considered in the context of the four questions the study was designed to answer. First, do the observed cutaneous responses represent true delayed

sensitivity reactions. Second, are the antigens capable of eliciting these responses tumour specific. Third, is skin test reactivity related to disease activity, and finally, does any correlation exist between tumour skin test reactivity and prognosis.

The positive cutaneous responses were clinically and histologically consistent with delayed sensitivity reactions exhibiting maximal induration of 5 mm or more and perivascular mononuclear cell deposition at 48 hr. The presence of some perivascular mononuclear cell accumulation in the site of clinically absent cutaneous reactivity noted in five instances was responsible for the lack of better correlation between clinical and histological readings in this study. The designation of 5 mm of induration as representative of delayed cutaneous sensitivity is an arbitrary one, chosen since most of our positive reactions fell within a 5–10 mm range of induration. The absence of a similar numerical criterion for establishing histological evidence of reactivity prompted us to use the clinical and not the histological, appearance of reaction sites for the purpose of this study.

The specificity of the observed reactions remains conjectural. (Seven patients, excluded from the study, did react to both ATE and ALE extracts.) The evaluable positive patients however, failed to respond to equipotent protein concentrations of autologous normal lymphocytes, This latter difference in reactivity may be due to a higher concentration of normal cell antigens in the tumour extracts or to the existence of tumour-specific antigens. In a similar study of patients suffering from a variety of malignant tumours (Oren & Herberman, 1971) the control extracts were found to contain at least as much HL-A antigen per mg protein as the tumour extracts.

There are several explanations for the lack of cutaneous response to tumour extract in both those patients with active disease and those in clinical remission. These include generalized anergy, weak or absent tumour antigenicity, the presence of a blocking antibody, or a direct and specific inhibitory effect of the viable tumour on this test of immunological reactivity. Ten of fifteen responders and eight of fifteen non-responders reacted to at least one of the battery of four skin test antigens used. Thus generalized anergy cannot explain the failure of response to tumour extract in over half the non-responders. The primary absence of tumour-specific antigenicity is another unlikely explanation since it could account neither for the loss of reactivity to tumour extract on the seven positive responders who relapsed nor for the reappearance of reactivity in the one patient of the seven who was retested following a second remission induction.

Circulating antibodies capable of protecting proliferating tumour cells from cytotoxic cellular immune reactions have been demonstrated in a variety of animal tumour systems (Hellstrom, Evans & Hellstrom, 1969; Hellstrom & Hellstrom, 1969; Heppner, 1969). Tumour protein extract from a positive responder in our study was incubated with serum from a non-responder while a similar amount of extract was incubated with saline. Although the saline-incubated extract gave a positive response upon intradermal challenge, the serum-incubated extract failed to elicit any reaction in the positive responder. This suggests the existence of blocking antibodies in the non-responder, and additional testing of this type (with suitable controls) is currently underway.

In 1966 Mikulska demonstrated that resistance to tumour autografts in rats was observed only after primary tumour removal (1966). He postulated that the immune response is limited and may be exhausted by the presence of a large tumour mass capable of attracting the sensitized lymphocytes. The findings in the present study are thus far consistent with this hypothesis. With one exception, all patients with clinically palpable tumour were skin

test negative. Those remaining negative even following remission relapsed earlier than those with a positive response to the autologous tumour extract, and all tested patients were negative at the time of relapse. The four positive reactions observed in patients with active cerebrospinal fluid disease, could be explained, according to this same hypothesis, by the failure of lymphocyte access to the subarachnoid space. A different mechanism for the apparent relationship between tumour mass and cutaneous reactivity to autologous tumour extract was proposed by Alexander *et al.* (1969) who observed histological hyperplasia of regional nodes draining a rat sarcoma associated with a failure of these nodes to release 'immunoblasts' following tumour autograft challenge. Since these same nodes reportedly released 'immunoblasts' following unrelated antigen stimulation, and since a response to tumour antigen stimulation was observed following extirpation of the primary tumour, Alexander postulated a specific inhibitory effect of the tumour on regional node release of 'immunoblasts' in response to tumour antigen challenge. According to the proposed mechanisms of both Mikulska and Alexander, reactivity to tumour extract may be a sensitive index of systemic disease activity. Oren & Herberman (1971) observed a similar correlation between cutaneous reactivity to tumour extracts and clinical status in patients with acute lymphoblastic leukaemia.

A significant association was noted in this study between reactivity to autologous tumour extract and prolonged remission. Do positive reactions simply indicate the absence of residual systemic tumour or might they represent a potent anti-tumour host response? The existence of such a response would provide a rationale for the use of immunotherapy through specific and non-specific stimulation that may lead to immunological control of this tumour.

ACKNOWLEDGMENTS

The authors wish to thank Mrs Sue Waymark for preparing the cellular extracts: Dr A. C. Templeton, Dr L. B. Thomas and Dr C. W. Berard for reviewing the histological sections; Mr Wilson Carswell for performing the biopsies and Mr Andrew Wamala for his secretarial assistance.

This work was supported by contract numbers PH-43-67-1343 and PH-43-67-67 of the National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

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