

Cellular immunity in progressive systemic sclerosis (scleroderma)

ALAN WINKELSTEIN,* GERALD P. RODNAN,† AND J. DANIEL HEILMAN‡
From the Department of Medicine, University of Pittsburgh, and the Montefiore Hospital and Presbyterian-University Hospital, Pittsburgh, Pennsylvania

Progressive systemic sclerosis (PSS) or scleroderma is a multisystem disease which is usually grouped with other systemic rheumatic or connective tissue disorders. As a class these conditions are believed to result, at least in part, from aberrant immunity (Rodnan, 1971). Previous studies have demonstrated that a significant percentage of affected patients develop a spectrum of autoantibodies, including antinuclear antibodies and rheumatoid factor (Rothfield and Rodnan, 1968; Beck, Anderson, Gray, and Rowell, 1963; Seligmann, Cannat, and Hamard, 1965). However, to date, there has been no systematic investigation of cell-mediated immunity in this disorder. The present investigation constitutes an evaluation of two parameters of this immune defence mechanism: the ability to manifest a cutaneous delayed hypersensitivity reaction and the *in vitro* lymphoproliferative response to the non-specific mitogen phytohaemagglutinin (PHA).

Materials and methods

Cutaneous delayed hypersensitivity was evaluated by intradermal testing with the following antigens:

Streptokinase-streptodornase, 0.1 ml. of a saline solution containing ten units of the former and 2.5 units of the latter;

Mumps, 0.1 ml.;

Candida 0.02 ml. of a 1:10 dilution (Hollister-Stier);

Trichophyton, 0.02 ml. (Hollister-Stier).

Skin tests were read at 24 hours and a reaction was considered positive if there was palpable induration in excess of 5 mm.

Lymphocyte responsiveness to PHA was measured by previously described techniques (Winkelstein and Craddock, 1967; Winkelstein, 1971). Two criteria of responsiveness were employed:

(1) Tritiated thymidine (H^3Tdr) incorporation as measured by DNA extraction with cold 5 per cent. trichloroacetic acid

(2) Percentage autoradiographic labelling.

For each patient, a minimum of three cultures was incubated with PHA (0.1 ml. Burroughs Wellcome) and two without added mitogens. Based on the number of lymphoid cells in the initial inoculum, counts were calculated as c.p.m./ 10^6 lymphocytes.

Patients studied

The 24 patients included in this study all had well-defined clinical evidence of PSS (Rodnan, 1971). All were evaluated for lymphocyte responsiveness to PHA and sixteen for cutaneous hypersensitivity. Cutaneous manifestations were classified as restricted (fingers, face) in ten and generalized in fourteen. No patient had significant azotaemia, nor were any receiving either adrenal cortical steroids or immunosuppressants at the time of study. All patients had total blood lymphocyte counts within the normal range.

Results

Sixteen patients were evaluated for cutaneous delayed hypersensitivity response employing a series of four antigens. This panel of antigens has been shown to elicit at least one positive response in a group of 37 normal controls (Table). All PSS patients tested demonstrated at least one positive response, indicating that the ability to manifest delayed hypersensitivity *in vivo* was intact.

In addition to assessing intactness of cutaneous delayed hypersensitivity, the lymphoproliferative response to PHA was evaluated in all patients (Figure). As can be seen, the incorporation of a pulse of H^3Tdr was quantitatively similar in the PSS patients and a group of 45 normal controls. These results were confirmed by autoradiography. The mean labelling index of patients with PSS was 33.4 ± 2.9 per cent. (S.E.), a value similar to that previously obtained in normal subjects (8).

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Address reprint requests to: Dr. Alan Winkelstein, M.D., Department of Medicine, Montefiore Hospital, Pittsburgh, Pennsylvania 15213, U.S.A.

* Assistant Professor of Medicine, University of Pittsburgh

† Professor of Medicine, University of Pittsburgh

‡ Chief Resident in Medicine, Montefiore Hospital

Table Positive delayed hypersensitivity skin tests in normals and patients with PSS

Antigen	Percentage responses	
	Normal subjects (37)	PSS patients (16)
SK-SD*	95	90
Mumps	90	85
Candida	40	32
Trichophyton	30	14
Percentage responding to at least one antigen	100	100

* Streptokinase-streptodornase

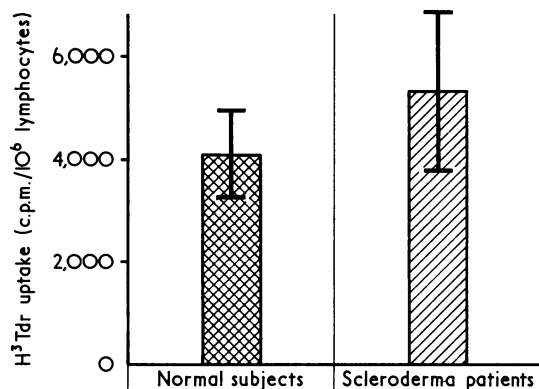


FIGURE H^3Tdr incorporation following PHA stimulation in patients with PSS and normal subjects. Results are corrected for the number of lymphocytes initially included in culture and expressed as c.p.m./ 10^6 lymphocytes. The vertical bars represent 1 standard error. No significant difference in the two groups was observed in this trial

Discussion

These studies demonstrate that by both *in vivo* and *in vitro* criteria, mechanisms of cell-mediated immunity are intact in patients with progressive systemic sclerosis (PSS). All patients responded with at least one positive skin test when challenged with a series of four non-specific antigens; this demonstrates that the mechanisms involved in this response *in vivo* are unimpaired by the disease. Furthermore, the proliferative response of lymphocytes to PHA *in vitro* was similar to that observed in a group of normal subjects. Although the exact significance of PHA

responsiveness is conjectural, it appears to correlate best with cellular immunity (Oppenheim, 1968); thus the normal response in these patients further substantiates conclusions regarding preservation of this immune defence mechanism.

Cell-mediated immune responses have become increasingly well recognized as an essential defense mechanism (Uhr, 1966). Impairment in delayed hypersensitivity has been associated with an increased susceptibility to infections with intracellular pathogens and a significant risk of malignant degeneration (Uhr, 1966; Hellström, Hellström, Evans, Heppner, Pierce, and Yang, 1969). Studies in patients with scleroderma suggest no increased incidence of malignancy (Calabro, 1967), nor are they unduly susceptible to viral, fungal, protozoal, or other intracellular infections (Rodnan, 1971). These data may be considered as strong clinical correlates substantiating an intact cellular immune defence system.

Although these results indicate no deficit in cellular immunity in PSS, they do not provide information regarding the possible patho-physiological role mediated by abnormal cellular immunity. Aberrations of this immune mechanism might contribute to the genesis of both the skin and systemic lesions. Further studies will therefore be necessary to determine whether certain limited responses participate in the development of PSS.

Summary

The integrity of cell-mediated immune defence mechanisms was assessed in a series of patients with progressive systemic sclerosis (scleroderma). Cutaneous delayed hypersensitivity responses were intact as assessed by at least one positive skin test after intradermal challenge with a panel of four antigens. The response *in vitro* to the non-specific mitogen phytohaemagglutinin, as measured by the incorporation of a pulse of H^3Tdr , was found to be similar to that observed in normal subjects. This response is believed to correlate primarily with other parameters of cellular immunity. Thus, by these two assays, no deficiency in the immune defence mechanism could be demonstrated in PSS.

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