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Studies of Mitogen Reactivity in Lymphocytes from Thermally Injured Patients (40893)[1]

RICHARD A. WINCHURCH, PARNELL KEELING, AND ANDREW M. MUNSTER

Department of Surgery, Johns Hopkins University School of Medicine and the Baltimore Firefighters Research Laboratory, Baltimore City Hospitals, Baltimore, Maryland 21224

Abstract. The *in vitro* reactivity of peripheral blood lymphocytes from burn patients to the T-cell mitogens phytohemagglutinin (PHA) and concanavalin A (Con A) was studied. Sequential studies showed that responses to PHA were either normal or elevated while responses to Con A were below normal and decreased further over the course of time. Analyses of patients' responses revealed no patterns of reactivity of prognostic value. However, the differences in reactivity to the T-cell mitogens PHA and Con A support the hypothesis that thermal injury produces specific rather than general effects on the immune system and lend credence to the concept that burns may exert a preferential influence on functionally distinct subpopulations of lymphocytes.

A clear assessment of the extent of immune dysfunction in burn patients could serve as a valuable adjunct for accurate prognosis and might provide a basis for the more effective administration of drug and immune therapy. Toward this end, a variety of methods have been used to test cellular immunity in these patients. These methods include the assessment of delayed cutaneous hypersensitivity to recall antigens and *in vitro* tests designed to measure responses to both antigens and soluble mitogens (1–4). A test system frequently employed in many laboratories is the measurement of T-lymphocyte reactivity to the mitogens phytohemagglutinin (PHA) and concanavalin A (Con A). These tests have been used widely in attempts to assess the severity of immune impairment following burn injury. However, previous reports on the reactivity of burn patients' lymphocytes to T-cell mitogens, particularly those concerning PHA, have indicated either normal or elevated lymphocyte responses and have left the predictive value of mitogen-induced responses open to question (3, 4). More recently, Baker and his colleagues (5) have reported that diminished responses to T-cell mitogens, particularly to PHA, were predictive of fatal sepsis in burn patients.

While the reactivity of lymphocytes to PHA and Con A does provide a means of monitoring T-lymphocyte reactivity, the cellular responses to these mitogens are not wholly equivalent. For example, it has been shown that different subpopulations of T lymphocytes respond to each of these mitogens (6). We have studied sequentially the responses of burn patients to PHA and Con A as well as to specific antigens. Our data indicate that neither PHA or Con A responsiveness *in vitro* can provide a clear basis for prognosis. There are essentially no clear differences in responsiveness to either of these mitogens in the populations of the patients who survived when compared with patients who succumbed to their injury. The data do show, however, that the impairment of the lymphocyte function is monitored more realistically by Con A responses than by PHA reactivity. Finally, these data support the hypothesis that burn injury produces specific rather than general effects on lymphocyte reactivity and that traumatic injury may have specific rather than general effects on immunity.

Materials and Methods. Lymphocyte cultures. Blood was drawn by venipuncture into heparinized syringes, mixed with an equal volume of saline, and 30 ml were layered over 10 ml of lymphocyte separa-

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tion medium (Litton Bionetics). After centrifugation at 400*g* for 20 min, the lymphocytes at the interphase were drawn off, washed three times with RPMI 1640 culture medium, and counted. Cultures were set in 0.2-ml volumes in cluster plates (Costar) and consisted of 3×10^5 responding lymphocytes in a complete medium composed of RPMI 1640 culture medium supplemented with 5% human AB serum (North American Biologicals), 50 units/ml penicillin, 50 μg/ml streptomycin, and 293 μg/ml L-glutamine (Grand Island Biologicals). Mixed lymphocyte culture contained additionally 1×10^5 mitomycin-treated stimulator lymphocytes from normal donors.

Mitogens and antigens. Concanavalin A (Con A) was obtained from Pharmacia Fine Chemicals and was used at a concentration of 2 μg/ml. Phytohemagglutinin (PHA-P) was obtained from Difco Laboratories and was used in a concentration of 25 μg/ml. Streptokinase–streptodornase (SKSD) was obtained from Lederle Laboratories and Mumps antigen was obtained from Microbiological Associates.

Culture conditions and assay procedure. Cultures were incubated for 3 days in the case of mitogens and for 7 days in the case of antigenic and MLC stimulation in a humidified atmosphere of 5% CO_2 in air at 37°C. Four hours prior to harvesting, each culture received 0.05 ml containing 0.5 μCi tritiated thymidine (spec. act. 2.0 Ci/mmole, New England Nuclear). Cultures were harvested using a semi-automated multiple sample harvester (Otto Hiller Co.) and the incorporation of a label into TCA-precipitable material was assessed by standard methods. Each experimental group consisted of at least four replicate cultures.

Results. The ages and the degree of injury of surviving and nonsurviving patients included in the study are presented in Table I. On the whole survivors were younger and suffered injury less severe than nonsurvivors. These data serve only to confirm the generally recognized fact that prognosis depends heavily on both the size of the burn and the age of the patient as well as on a combination of these and other factors.

Table II summarizes the data from experiments where the reactivity of burned patients' lymphocytes to the T-cell mitogens PHA and Con A was tested. Additionally, responses to representative antigens and to allogeneic cells are also shown. These data are pooled from a number of experiments performed at different intervals after injury. A representative sample of data from normal healthy controls is presented for comparison. These data show that the average of patients' responses to PHA does not differ from the normal response to this mitogen. On the other hand, the patients' responses to Con A are substantially depressed when compared with normal Con A-induced responses. The patients' reactivity to antigenic stimulation and in mixed lymphocyte culture are also depressed and these specific immune responses appear to be more closely correlated with the Con A responses than with the PHA responses. The disparity in mitogen-induced reactivity seems to indicate a differential susceptibility of Con A-reactive lymphocytes to the suppressive effects of thermal injury. However, it might be argued that differences in pooled data such as those presented in Table I are artificial and that both PHA- and Con A-induced responses could either increase or decrease similarly over the course of time. Thus it might be possible that while the depression in reactivities observed were quantitatively different, they could be qualitatively similar and follow similar kinetic patterns. Thus, we reanalyzed the

TABLE I. AGES AND DEGREE OF INJURY OF THE PATIENT POPULATION UNDER STUDY

	Age[a]	Range	Percentage burn[a]	Range
Fatalities	59 ± 5	(24–87)	44 ± 6	(25–86)
Survivors	26 ± 3	(16–46)	29 ± 4	(20–48)
Total	46 ± 5	(16–87)	38 ± 4	(20–86)

[a] Mean ± SEM.

TABLE II. COMPARISON OF *IN VITRO* RESPONSES OF NORMAL SUBJECTS AND BURN PATIENTS TO ANTIGENS AND MITOGENS, AND IN MIXED LYMPHOCYTE CULTURES

	Tritiated thymidine incorporation, cpm/culture ± SE		
	Normal donors	Patients	P^a
PHA 25 μg/ml	61,980 ± 19,674 $n = 3$	57,393 ± 6,094 $n = 38$	NS
Con A 2 μg/ml	59,261 ± 16,054 $n = 5$	13,995 ± 3,084 $n = 38$	0.001
SKSD 120 units/ml	28,404 ± 8,470 $n = 8$	3,055 ± 1,012 $n = 16$	0.01
Mumps 12.5^{-1} dilution	26,000 ± 6,677 $n = 8$	1,653 ± 298 $n = 15$	0.001
Mixed lymphocyte culture	17,206 ± 3,300 $n = 7$	4,666 ± 934 $n = 14$	0.002

[a] Students t test.

data by plotting the patients' responses to both mitogens from the early postburn period and at weekly intervals up to 4 weeks after injury. These data, which are presented in Fig. 1, show that the response of patients' lymphocytes to Con A in the early postburn period are still dramatically lower than control responses and that the patients' responses decline progressively over the next 4 weeks. On the other hand, patients' responses to PHA were equivalent to normal responses during the early postburn period, increased somewhat by 1 to 2 weeks postburn, and by 4 weeks, had returned to normal levels. In Fig. 2, these same patients' responses were further divided into two categories: (a) patients who survived and (b) patients who eventually succumbed to their injury. In both groups, Con A responses decreased progressively over the course of time. PHA-induced responses increased dramatically at about 1 week postburn and subsequently declined while the responses of patient fatalities increased gradually over the next 2 weeks postburn and remained at high levels at 4 weeks.

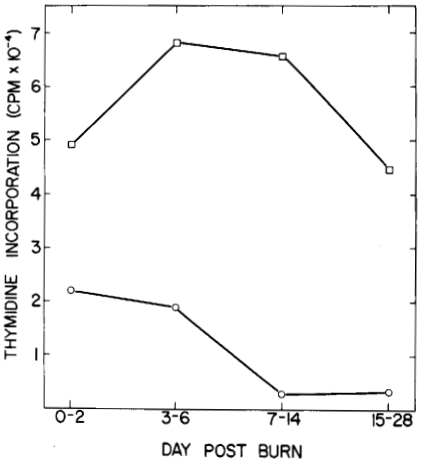

FIG. 1. Sequential measurement of mitogen responses in lymphocytes from burn patients. (□——□) PHA responses. (○——○) Con A responses.

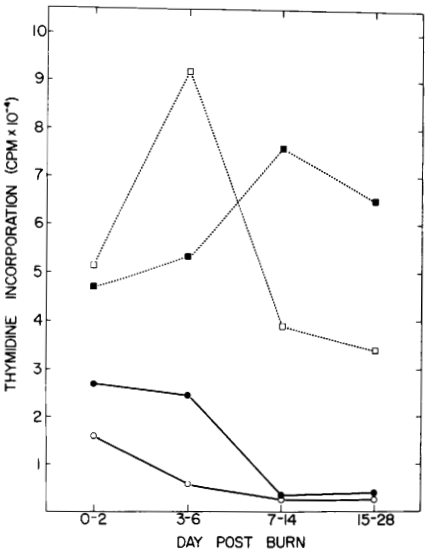

FIG. 2. Sequential measurement of mitogen responses in lymphocytes from surviving and nonsurviving burn patients. (●——●) Con A responses and (■- -■) PHA responses in burn fatalities. (○——○) Con A responses and (□- -□) PHA responses in survivors.

Discussion. Two conclusions can be drawn from the present data. First, the reactivity of burned patients' lymphocytes to the T-cell mitogens Con A and PHA provides no firm basis for prognosis. Despite the disparity between the PHA- and Con A-induced reactivity, the eventual outcome, either survival or death, cannot be predicted from the reactivity to either mitogen. Second, the present data support the concept that burn injury produces specific rather than general effects on the host lymphocytes. In other words, burn injury appears to depress the function of some subsets of T lymphocytes dramatically while the function of other subpopulations of cells remain unaffected. While PHA responses in almost all cases and over the course of an interval of 4 weeks after injury are either normal or elevated, Con A responses are depressed. In terms of providing a model system for monitoring the immune capability of patients, the PHA assay is inadequate. On the other hand, Con A-induced responses reflect the general pattern of reactivity of burned patients' lymphocytes to *in vitro* antigen stimulation (7) and appeared to provide a better model system by which the adequacy of host immune defenses can be measured. More importantly, however, is the fact that the disparity between Con A and PHA reactivity lends credence to the concept that burn injury may preferentially affect functionally distinct subpopulations of T lymphocytes such as T-helper or -suppressor cells. Reports from several laboratories including our own (8–10) have indicated that burn injury is associated with disproportionate increases in T-suppressor cells and that these cells may account for the depressed cellular immunity associated with burn injury.

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