Longitudinal Assay of Lymphocyte Responsiveness in Patients with Major Burns

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Serial blast transformation *in vitro* was measured in peripheral lymphocytes from 38 patients with major thermal injury. Lymphocytes were tested with the antigens streptokinase-streptodornase (SKSD), mumps and purified protein derivative (PPD), the mitogens conconavadin A (Con A) and phytohemagglutinin (PHA), and in the one-way mixed lymphocyte reaction. Statistically significant suppression by the burn injury was noticed in all measurements except response to PHA. One-time measurements were not significantly different between the patients who survived and the patients who did not survive their burn injuries. However, serial determinations of responsiveness to the three natural antigens SKSD, mumps and PPD, as well as the mixed lymphocyte reaction accurately reflected prognosis.

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IMMUNOLOGIC ALTERATIONS in patients who have been burned have received increased attention since the early classic work of Alexander and Moncrief.[1] In particular, suppression of cell-mediated immunity has been documented by several reports in both man and laboratory animal models. Blast transformation of peripheral lymphocytes, in response to the phytomitogens Phytohemagglutinin (PHA) and Concanavalin A (Con A), has produced controversial findings in the literature. Eurenius and Mortensen,[4] Daniels,[3] and Mahler and Bachelor[5] have reported increased responsiveness to pHA, while Baker[2] and Rittenbury[10] have reported decreased responsiveness to PHA. This report involves the longitudinal testing of the peripheral lymphocyte responsiveness of a group of burn patients over a period of several weeks. Using the mitogens PHA and Con A, the antigens streptokinase-streptodornase (SKSD), mumps, and purified protein derivative (PPD), as well as the mixed lymphocyte reaction, we attempted to map the natural history of lymphocyte transformation in these patients.

Materials and Methods

Clinical Material

Thirty-eight patients with a mean burn size of 38% of the total body surface were included in the study. The age range was between 18 and 80 years, and there were 22 deaths and 16 survivors.

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Lymphocyte Cultures

Blood was drawn by venipuncture into heparinized syringes, mixed with an equal volume of saline solution and 30 ml were layered over 10 ml of lymphocyte separation medium (Litton Bionetics). After the blood samples were centrifuged at 40 *g* for 20 minutes, the lymphocytes at the interface were drawn off, washed 3 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 per ml penicillin, 50 μg per ml streptomycin, and 293 μg/ml L-glutamine (Grant Island Biologicals). Mixed lymphocyte cultures contained, additionally, 1×10^5 mitomycin-treated stimulator lymphocytes from normal donors.

Mitogens and Antigens

Con A was used in a concentration of 2 μg per ml. PHA was used in a concentration of 25 μg/ml.

Culture Conditions and Assay Procedure

Mitogen cultures were incubated for three days, and antigen and MLC stimulation cultures were incubated for seven days in a humidified atmosphere of 5% CO_2 in air at 37 C. Four hours before harvesting, each culture received 0.05 ml of a solution containing 0.5 millicuries Thymidine treated with tritium. The cultures were harvested using a semiautomated multiple sample harvester (Otto Hiller Co.), and the results

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TABLE 1. *Antigenic Responsiveness of Peripheral Lymphocytes from Burn Patients, Disintegrations Per Minute*

Antigen and Dose	Patients	Normal Controls	p
SKSD 4 units	2,694 ± 584	8,292 ± 3,732	<0.01
SKSD 120	6,214 ± 1,107	28,404 ± 8,470	<0.001
PPD 2.5	4,335 ± 1,046	11,872 ± 3,568	N.S.
PPD 10	5,069 ± 1,128	30,362 ± 8,709	<0.05
Mumps 1/18	4,306 ± 723	19,838 ± 5,216	<0.001
Mumps 1/12	4,836 ± 800	23,772 ± 7,044	<0.001
MLC	7,524 ± 976	17,206 ± 3,300	<0.01
Con A	13,995 ± 3,084	59,261 ± 16,054	<0.001
PHA	57,373 ± 6,094	61,980 ± 19,674	N.S.

of the addition of a radioactive isotope label into TCA-preciptable material were assessed by standard methods. Each experimental group consisted of at least four replicate cultures.

Results

Table 1 shows the antigenic responsiveness of peripheral lymphocytes from the patients compared with 20 normal controls. There was a statistically significant suppression of antigenic response in the burn patient group, except for phytohemagglutinin responsiveness, where there is no difference, and for PPD in dosages of 2.5 μg, which were still depressed, but where the large standard error in the controls precluded statistical significance.

Table 2 shows the responsiveness of the patients who survived and the patients who died to each of the antigens and are represented as pooled data. There is no statistically significant difference between any of the patients who survived and the patients who died.

Figures 1 through 4 illustrate lymphocyte reactivity when plotted longitudinally over a period of time according to lines of best fit. Figure 1 illustrates reactivity to SKSD; Figure 2 illustrates reactivity PPD; Figure 3 illustrates reactivity to mumps; and Figure 4 illustrates reactivity to PHA, Con A, and the mixed lymphocyte reaction. Although a statistically significant correlation is not achieved in all these regression lines, largely because of the large standard error inherent in lymphocyte blast transformation measurements over a period of time, in the patients who died a significant downward regression is noticed in the responsiveness to SKSD (4 units), PPD (2.5 and 10 units), mumps (dilution of 1 in 18), the mixed lymphocyte reaction, and Con A. A significant downward regression was obtained for Con A in patients who survived, and a significant upward regression was obtained in phytohemagglutinin responsiveness

TABLE 2. In Vitro *Antigen-induced Responses of Burn Patients, Pooled Data for all Samplings Postburn**

	Antigen Dose	Patients	
		Survivors	Deaths
Mumps	1/18	4,656 ± 889	4,416 ± 1,056
(Dilution)	1/12	5,342 ± 952	4,707 ± 1,214
PPD	2.5	2,286 ± 889	5,469 ± 1,681
	10	2,777 ± 640	6,816 ± 1,857
SKSD	4	2,531 ± 607	2,711 ± 875
	120	5,480 ± 994	6,748 ± 1,785
Mixed lymphocyte cultures†	—	7,243 ± 1,485	8,135 ± 1,400
PHA		74,016 ± 11,061	67,556 ± 8,722
Con A		6,710 ± 3,940	11,898 ± 4,202

* Mean CPM tritiated thymidine incorporation ± SEM.

† MLC containing 3×10^5 responder lymphocytes and 1×10^5 mitomycin-treated stimulator lymphocytes.

All groups, survivors vs deaths NS by t-test.

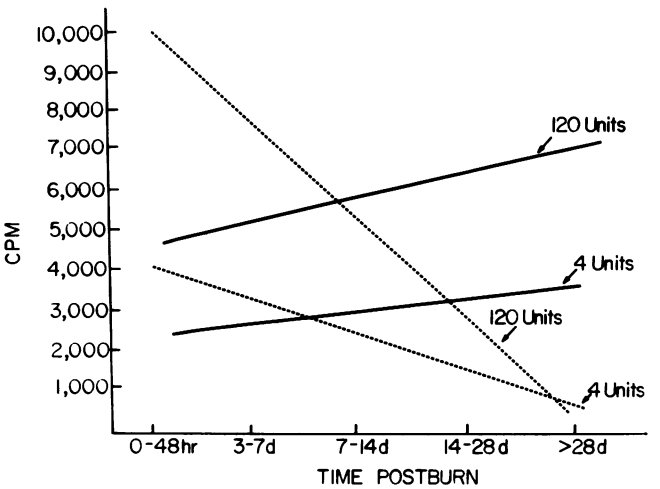

FIG. 1. Lymphocyte reactivity to SKSD. $r = -0.900$, $p < 0.05$ for SKSD 4 units in nonsurvivors, remainder NS. Solid line: survivors. Dotted line: nonsurvivors.

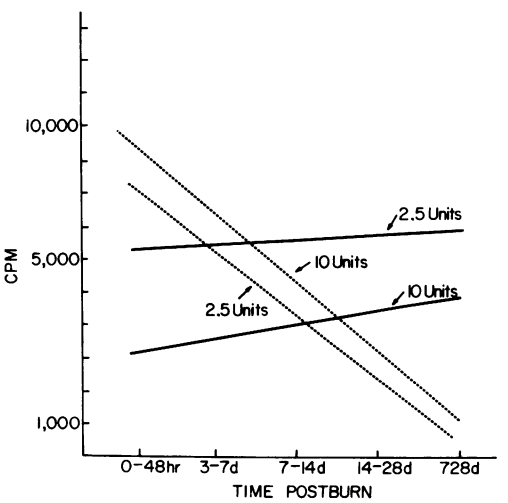

FIG. 2. Lymphocyte reactivity to PPD $r = -0.955$ for PPD 2.5 and -0.933 for PPD 10, both in nonsurvivors, $p < 0.01$. The line in survivors NS. Solid line: survivors. Dotted line: nonsurvivors.

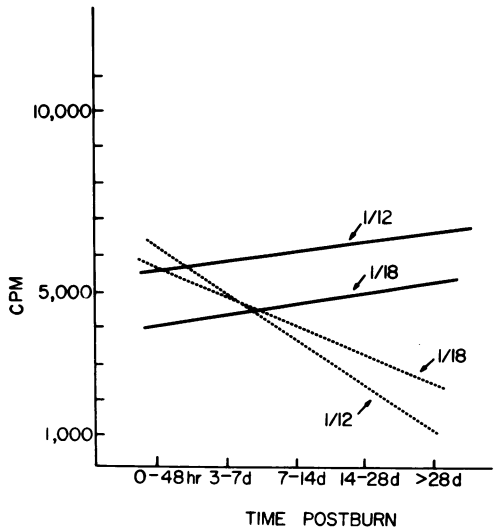

FIG. 3 Lymphocyte reactivity to mumps. $r = -0.793$, $p < 0.05$, for mumps 1 in 18 dilution, nonsurvivors, remainder NS. Solid line: survivors. Dotted line: nonsurvivors.

in patients who died. Thus the statistical analysis reveals a significant prognosis of nonsurvival using 4 units of SKSD, PPD, a 1 in 18 dilution of mumps and the mixed lymphocyte reaction; Con A was an accurate mirror of the burn injury, but was statistically unable to differentiate between survivors and nonsurvivors; and phytohemagglutinin reactivity appeared to increase in the nonsurvivors with the progression of time.

Discussion

It is important to establish the natural history of immunologic changes following disease and surgical injury, both for the identification of and prognosis for at risk groups, and for the timing of immunologic interventions such as vaccination or immunomodulation. Skin testing to measure delayed hypersensitivity antigens has been popularized by the McGill group,[9] but there is evidence that repeated skin testing may alter the subsequent responses of patients. In any event, repeated skin testing with a variety of antigens may be impractical in an extensively burned individual. Our results show that serial testing of blast transformation *in vitro* is an accurate predictor of survival, but that to be of significance these responses have to be measured serially since neither pooled data nor one-time measurements showed a statistically significant difference between the survivors and the nonsurvivors. We also confirm our own and other investigators earlier observations that phytohemagglutinin responsiveness is practically meaningless both in the documentation of the effect of injury and as a prognostic tool.

Blast transformation of peripheral lymphocytes *in vitro* is not a pure test of T-cell responsiveness, since there is a minimum requirement for a macrophage population for blast transformation to occur. Since, however, neutrophil migration, macrophage migration and the inflammatory reaction are not involved in blast transformation, *in vitro* these results can be interpreted somewhat more precisely than the results of skin testing.

There are one or two other interesting observations that may be made from these results. Although lack of statistical significance between one-time measurements precludes any firm conclusions, inspection of Figures 1 to 4 reveals that, as a rule, the reactivity of lymphocytes from patients who eventually died actually started at a higher level than that of those who eventually survived. That means that in the time course of the injury there comes a "Crossover point" at which time the reactivity of the survivors, rising with the progression of time, crosses the line of reactivity of the nonsurvivors, which fall with the progression of time. This crossover point varies according to the antigen being tested, occurring as early as 48 hours in response to a 1 in 18 dilution of mumps antigen, or as late as 14 days in the case of 10 units of PPD. If the development of immunosuppression following burn injury is an effect of suppressor cell stimulation, as was postulated first by us[7] and now widely demonstrated,[6,8] then it is possible that these suppressor cells clone at different times after the injury, according to which antigen is being looked at. This finding would dictate great caution in the use of immunomodulating agents which have a nonspecific action on host defenses, where the time of administration may be critical. For this reason we have incorporated the testing of immunomodulating agents

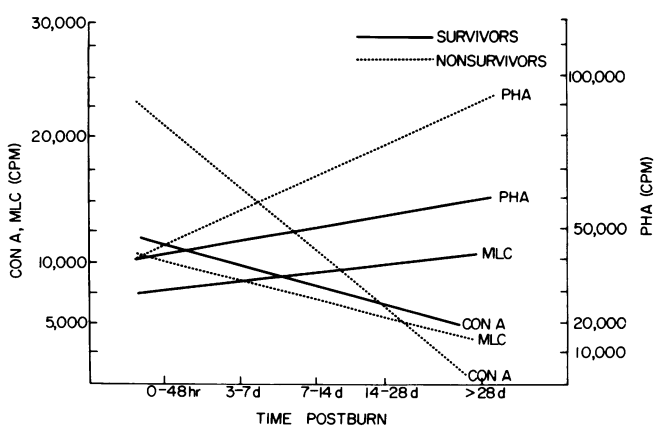

FIG. 4. Lymphocyte reactivity to PHA, Con A, and in MLC. $r = -0.744$, $p < 0.05$ for MLC in nonsurvivors, $r = -0.911$, $p < 0.01$ for Con A in nonsurvivors, $r = -0.801$, $p < 0.05$ for Con A in survivors, $r = 0.888$, $p < 0.05$ for PHA nonsurvivors, NS for remainder. Solid line: survivors. Dotted line: nonsurvivors.

into this *in vitro* system; these results will be reported in the future.

In conclusion, *in vitro* blast transformation of human peripheral lymphocytes following thermal trauma is an accurate predictor of outcome provided serial determinations are carried out; the most accurate indicators of prognosis are the natural antigens, and the least accurate, the mitogen PHA.

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