Immunological Incompetence in Burn Patients*

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SUMMARY Immunological competence was evaluated in 51 burn patients, aged 8½ months to 73 years. The studies included introblue tetrazolium dye reduction test (NBT) for intracellular killing activity of phagocytes and immunoglobulin levels (IgG, IgA, IgM) for humoral immunity. The cell-mediated immunity was determined *in vivo* by delayed hypersensitivity skin test (DHST) to three recalled antigens and *in vitro* by phytohaemagglutinin (PHA) stimulated blast transformation of peripheral blood lymphocytes. A significant reduction in IgG, IgA and IgM levels was found in 74.4, 24.4 and 7.7 per cent of the patients, respectively. The abnormally low levels of immunoglobulin correlated with the amount of time elapsed after the burn (for IgG 88.9 per cent of the patients in the first week) and with the extent of burn area (for IgG 100 per cent of the patients with burns covering more than 40 per cent of the body) but there was no correlation with the patients' age. Abnormal NBT was found in 15.5 per cent of the patients; only 4.2 per cent of them showed abnormal cell-mediated immunity. Impaired immunological status in burn patients correlated well with the incidence of infections. Wound sepsis and septicaemia were the main cause of death in four out of five patients who died and all of them had low IgG levels, three had low IgA levels, two had abnormal DHST and one had abnormal NBT and PHA stimulation test.

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Since the refinement of modern fluid resuscitation and respiratory care techniques, sepsis following thermal injury has emerged as the major cause of mortality.[1,2] The high incidence of fatal septicaemia associated with severe thermal injury is believed to result from impairment of immunocompetence. A variety of *in vivo* and *in vitro* assays suggest that various parameters of immune functions are compromised by thermal injury.[3,4] Both non-specific and specific immunity are affected including the impairment of chemotaxis,[5] phagocytosis,[6] complement,[7] humoral immunity[8] and cell-mediated immunity.[9] This report described an abnormality in the intracellular killing activity of phagocytes, immunoglobulin levels and cell-mediated immunity in burn patients studied at Siriraj Hospital, Bangkok, Thailand.

MATERIALS AND METHODS

Patients

Fifty-one burn patients, ranging in age from 8½ months to 73 years with a male to female ratio of 2:1, were studied for immunological competence as soon after injuries as possible. All had been admitted to the Burn Unit, Department of Surgery, Siriraj Hospital, suffering from second and third degree burns covering between 10 and 85 per cent of the body surface area (BSA) (average 31.7 per cent). They were treated using the standard regimen of the Burn Unit. Treatment included rigorous resuscitation with crystaloids such as Ringer's lactate or hypertonic salt solutions, immediate care of the airway, escharotomy in cases of deep circumferential burns, and other general symptomatic measures. Each patient received routine topical therapy and systemic antibiotics upon specific indications. Blood and blood products were given only when there were definite indica-

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tions. The studies included nitroblue tetrazolium dye reduction test (NBT) for intracellular killing activity of phagocytes and immunoglobulin level (IgG, IgA, IgM) for humoral immunity. Cell-mediated immunity was determined *in vivo* by delayed hypersensitivity skin test (DHST) to three recalled antigens and *in vitro* by phytohaemagglutinin (PHA) stimulated blast transformation of the peripheral blood lymphocytes.

Nitrobule tetrazolium dye reduction test

The method used for both the resting and stimulated NBT was that of Park *et al;*[10] results were expressed using the graph developed by Feigin *et al.*[11] Normal controls were obtained from 20 herltly children and 35 herltly adults. In the endotoxin stimulated NBT, the number of positive cells less than 20 per cent and an absolute number less than 1,000 were considered abnormal.

Immunoglobulin levels

Determinations of the concentration of IgG, IgA and IgM were carried out using the radial immunodiffusion method of Mancini *et al,*[12] using commercially available radial immunodiffusion plates (Hyland, Division of Travenol Laboratories, Inc., U.S.A.). The results were considered abnormally low when the immunoglobulin levels were less than mean minus 2 standard deviations of normal levels in Thais of the same age group.[13]

Cell-mediated immunity

The cell-mediated immune response (CIMR) was studied *in vivo* by delayed hypersensitivity skin test (DHST) to three recalled antigens including purified protein derivative (PPD) tuberculin 5 T.U., candida 1:100 (Center Laboratories, U.S.A.) and trichophyton 1:100 (Center Laboratories, U.S.A.), injected intracutaneously and the results were read at 48 hours. The reaction with an induration greater than 5 millimetres was considered to be positive. A patient who had a positive skin reaction to at least one antigen tested was considered to have positive DHST (normal *in vivo* test). CMIR was also studied *in vitro* by stimulation of Ficoll-Hypaque separated peripheral blood lymphocytes[14] with PHA in tissue culture for three days; the percentage of blast transformation was determined by using the method previously described.[15,16] In addition, DNA synthesis in these blast cells was determined by tritiated thymidine incorporation; the radioactivity was measured by liquid scintillation counter. A percentage of blast transformation of less than 70 per cent and the tritiated thymidine uptake of less than 150,000 dpm (disintegration per minute) were considered to be abnormal (the normal value of blast transformation determined from tests on 35 normal controls was 81.2±7.1 per cent; ^{3}H-thymidine uptake, 553,896±42,652 dpm).

RESULTS

Among 43 burn patients who had complete immunoglobulin determinations, 74.4 per cent had abnormally low IgG levels; 24.4 per cent, abnormally low IgA; and 7.7 per cent, abnormally low IgM. The correlation between abnormal immunoglobulin levels and the age of burn patients is shown in Table 1. In a comparison of 20 children and 23 adults it may be seen that there is no significant difference in the percentage of patients who had abnormal immunoglobulin levels. Table 2 shows the correlation between abnormal immunoglobulin levels and the amount of time which lapsed after the burn. The majority of patients (88.9%) had abnormally low IgG during days 1-7 after burn; the number fell to 50 per cent during days 8-14 after burn and declined to only 25 per cent 14 days after burn. Similarly, abnormal IgA and IgM levels occurred during the first week after burn. The correlation between abnormal immunoglobulin levels and the extent of burn areas and the time lapse following the burn (less than seven days and more than seven days) is shown in Table 3. As the extent of the burn area in individual patients increased compared with others, there was a cor-

Table 1 Correlation of abnormal immunoglobulin levels with age of the patients.

Age (yrs)	No. of patients	% abnormal IgG	IgA	IgM
⩽15	20	75	26.3	10.5
>15	23	73.9	22.7	5
Total	43	74.4	24.4	7.7

Table 2 Correlation of abnormal immunoglobulin levels with the time elapsed after the burn

Days after burn	No. of patients	% abnormal IgG	IgA	IgM
1-7	27	88.9	38.5	8
8-14	12	50	0	11.1
>14	5	25	0	0

respondingly slight increase in the percentage of patients who had abnormal IgG levels, but these differences were not statistically significant. However, there was no correlation between abnormal IgA and IgM levels and the extent of the burn areas.

Table 4 shows the results of intracellular killing activity of phagocytes and cell-mediated immunity in 45 burn patients. Among 16 patients who had negative skin tests, 12 were children less than 13 years of age and one adult patient 52 years old. However, neither the age of the patients, the amount of time elapsed after thermal injury nor the extent of the burn correlated with abnormal PHA stimulation of peripheral blood lymphocytes.

Table 5 shows the immunological status of five fatal burn cases. All were adult patients except one, and two of the adults were old. Four out of the five patients died from wound sepsis and septicaemia, the micro-organisms identified were *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.* They died during the late stage, ranging from 10 to 58 days after the burn. All the patients who died from sepsis had at least one abnormal immunological test but the last patient, who died from shock resulting from acute diarrhoea, had normal immunological functions. A 52-year-old man who had a 40% BSA burn, had abnormal immunological tests in all instances except for the IgM test. He died from septicaemia 10 days after the burn.

Table 3 Correlation of abnormal immunoglobulin levels with the extent of burn areas and time elapsed after burn

% BSA	No. of patients	% abnormal IgG ⩽7d	IgG >7d	IgA ⩽7d	IgA >7d	IgM ⩽7d	IgM >7d
1-20	15	81.8	25	30	0	11.1	0
21-40	19	90	66.6	60	0	10	12.5
>40	9	100	0	16.6	0	0	0

BSA = body surface area
d = days after burn

Table 4 Intracellular killing activity of phagocytes and cell-mediated immunity in 45 patients.

	No. of tests	No. abnormal	% abnormal
NBT	45	7	15.6
PHA stimulation	48	2	4.2
DHST	49	16*	32.6

*Negative skin test to three antigens

DISCUSSION

Burn injuries result in complex alterations in host defense mechanisms and it is likely that such an impairment allows burn wound sepsis to advance to septicaemia with its fatal consequences. These include deficits in neutrophil and lymphocyte functions,[17,18] anergy to delayed hypersensitivity skin tests,[19] depletion of immunoglobulins,[20] alterations of the complement cascade,[7,21] and functional depression of the reticulo-endothelial system.[6,22] In the present study, the most striking abnormality was the abnormally low immunoglobulin levels, especially those of IgG.

Table 5 Immunological status of five fatal cases

Sex, age in yrs	% BSA	NBT	IgG	IgA	IgM	PHA stimulation	DHST	Days after burn	Causes of death
F, 21	62	N	AB	AB	N	N	N	12	Wound sepsis, septicaemia
F, 2	25	N	AB	AB	N	N	AB	26	Wound sepsis
M, 52	40	AB	AB	AB	N	AB	AB	10	Septicaemia
M, 60	46	N	AB	N	N	N	N	58	Septicaemia, pneumonia
F, 30	28	N	N	N	N	N	–	22	Diarrhoea, shock
Immunological abnormalities (%)		20	80	60	0	20	50		Infection = 100

N = normal, AB = abnormal

The reduction of IgG levels occurred mostly during the first week after the burn, but the IgA and IgM levels were less affected. These abnormalities were probably due to the leakage of IgG along with other plasma protein through the burn area since abnormally low IgG levels correlated well with the extent of the burn area but it was less striking for IgA and IgM. Explanations for the apparently undisturbed IgG levels in the group of patients with BSA over 40 per cent and time lapse exceeding seven days after the burn, include possible IgG restoration following secondary infections, and the wariability due to too small number of patients. These findings are in agreement with those of previous studies. In a serial analysis of serum immunoglobulin profiles in 50 burn patients, Munster *et al*[20] found severe depression of IgG concentration during the post-burn period, rising to a normal level within one to two months although the levels of IgA and IgM remained relatively unaltered. However, Arturson *et al*[23] found a decrease in all serum immunoglobulins (IgG, IgA, IgM, IgD and IgE) two days after the burn injury of 15 adults. The immunoglobulin levels reached a nadir five days after the burn and returned to normal by the end of the second week. Bjornson *et al*[24] also described reduced serum IgA and IgG levels during the first 10 days after the burn as well as decreased IgM levels from three to six weeks post-burn. It was concluded that mechanical leakage of protein from the burn wound does not entirely account for the pattern of abnormalities observed, but that many factors may be involved such as synthesis, catabolism, and redistribution of fluid and protein between oedema and intravascular spaces, etc.

Using the nitroblue tetrazolium reduction test (NBT), abnormal intracellular killing activity of phagocytes was found in 15.6 per cent of patients. The abnormal NBT did not correlate with the extent of burn area or the amount of the time elapsed after the burn, but it seemed to correlate well with the occurrence of sepsis. All patients having abnormal NBT had wound sepsis and one patient died from severe septicaemia. These findings confirm the previous report by Curreri *et al*[25] who used NBT to assess neutrophil function for predicting wound sepsis in burn patients. Other abnormal functions of neutrophil and macrophage in burn patients had been investigated. Fikrig *et al*[5] demonstrated decreased chemotaxis by polymorphonuclear leukocytes in seven out of 22 burn patients. Opsonin and opsonic glycoprotein (plasma fibronectin) levels were depleted soon after burn injury.[7,22,26] Alexander[4] also reported decreased opsonins in the serum of patients with severe burns,[4] and decreased intracellular killing of the phagocytosed bacteria (*Staphylococcus aureus* 502 A).[17] The decreased bacterial killing activity of the peripheral blood neutrophils was attributed to their diminished specific granules,[27] and decreased neutrophil concentrations of lysozyme, acid phosphatase and beta-glucuronidase.[3,28]

Suppression of cell-mediated immunity in burn patients as determined by the *in vivo* test (DHST) and *in vitro* test (PHA stimulation) was found in 32.6 per cent and 4.2 per cent of patients, respectively. This discrepancy may be due to the fact that many young children, who had a negative skin test, may not have had the chance to be exposed to the three antigens used in the delayed hypersensitivity skin test, but they exhibited normal blast transformation after stimulation with PHA *in vitro*. Impaired cellular immunity following thermal injury has long been observed clinically as anergy and prolonged survival of skin allografts as well as xenografts.[9,19,29-31] During the early post-burn period, patients usually have decreased skin reactivity to recalled antigens such as streptokinase-streptodornase, mumps, tuberculin, diphtheria toxoid and *Candida*. Anergy also correlates with coexistent impairment of patients' peripheral blood lymphocyte activation by PHA and other specific antigens (i.e. streptokinase-streptodornase, mumps, PPD tuberculin and in the one-way mixed lymphocyte reaction).[32] Miller[33] showed a decreased PHA response preceding sepsis by four to six days, while patients who were infected but did not progress to septicaemia had an increased PHA response.[33] Several reports suggest that the burn patients' serum suppresses the PHA responsiveness of normal lymphocytes as well as mixed-lymphocyte reaction (MLR) and that impaired delayed hypersensitivity skin tests and skin allograft survival are directly related to the immunosuppressive activity of the patients' serum.[9,34-36] Wolfe *et al*[34,37] found that the presence of a serum suppressor either preceded or coincided with the onset of impaired lymphocyte responsiveness to PHA. This immunosuppressive serum activity is found in a polypeptide fraction of low molecular weight (10,000 Daltons),[35] and it is found in both patients' serum and blister fluid.[38] However, subsequent study indicated that the presence of immunosuppressive activity in burn serum did not correlate with the degree of lymphocyte hyporesponsiveness to PHA and may, instead, be due in part to the emergence of a suppressor T-cell population.[39] This hypothesis was supported by the discovery of circulating suppressor lymphocytes in burn patients.[40-42] Further studies of T-cell subpopulations following thermal injury, either by using Fc recepters of different immunoglobulin classes as surface markers for Tμ (helper cells) and Tγ (suppressor cells) or by using monoclonal antibodies for detection of helper/inducer (OKT4) and suppressor/cytotoxic (OKT8) T cells in the peripheral blood, showed that severe burn injuries regularly induce an early transient increase

in circulating suppressor cells accompanied by a depression in lymphocyte activation.[43,44] An inversion of the normal ratio between suppressor and helper T-cell occurred soon after burn injury, reached a peak in five to seven days and then returned gradually to normal levels by 14 days after the burn. High levels of circulating suppressor cell activity later in the post-burn course accurately predict mortality from sepsis.[44] It was concluded that cell-mediated immunity is suppressed soon after thermal injury. This suppression is associated with, and may be caused by, a relative increase in the percentage of circulating suppressor T lymphocytes. A suppressive factor, perhaps a product of suppressor cells, appears in the serum. It has been suggested that immunosuppression observed in severely burned patients appears as a defense against autoimmunity which might otherwise result from intense tissue antigenic bombardment after injury.[3,34,44]

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