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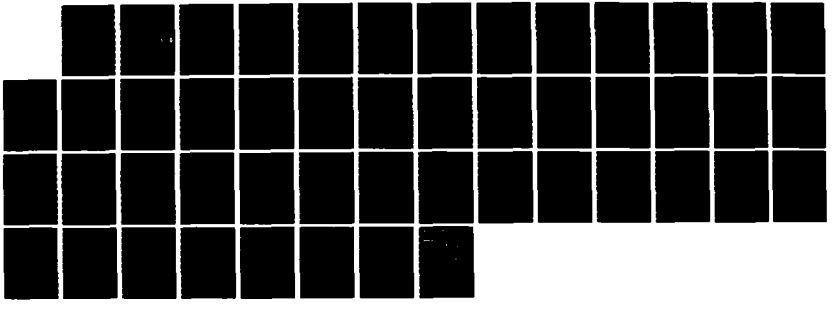

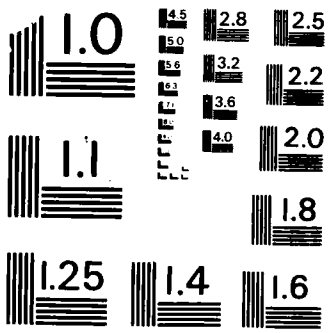

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Host Defense Against Opportunist Microorganisms Following Trauma

ANNUAL SUMMARY REPORT

Ann B. Bjornson, Ph.D.
H. Stephen Bjornson, M.D., Ph.D.
Josef E. Fischer, M.D.

September 27, 1984

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The temporal occurrence of multiple immunologic alterations resulting from thermal injury was investigated in guinea pigs with scald burns of 30% total body surface. Groups of 2-4 burned and sham-treated animals were sacrificed at 1-3 day intervals during 3 weeks postburn. C3 concentration, total and alternative complement pathway-mediated C3 fixation on Pseudomonas aeruginosa, proportion of native C3 to C3 cleavage fragments, and prostaglandin (PG) E_2 level were measured in serum or plasma. Bactericidal activity of peripheral polymorphonuclear leukocytes (PMN) against P. aeruginosa, proliferative responses of splenic lymphocytes to phytohemagglutinin and concanavalin A, and clearance of radiolabeled P. aeruginosa by the reticuloendothelial system were also determined. Complement consumption with concomitant reduction in C3 concentration and bacterial C3 fixation, and elevation of PGE_2 occurred within 3-6 h postburn. These alterations were accompanied by reduction in intrinsic PMN bactericidal activity,

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suppression of PMN bactericidal activity by serum, and a minor decrease in blood clearance of P. aeruginosa. Complement and PMN dysfunction returned to normal by the end of the first week postburn. A clear temporal separation in the occurrence of depression in lymphoproliferative responses was evident, since this alteration was not observed until 4 days postburn and was maximal during 7-9 days postburn. Our results support the concept that there is a continuum of immunologic alterations resulting from thermal injury and that arachidonate metabolites and complement cleavage fragments participate in its initiation.

HOST DEFENSE AGAINST OPPORTUNIST MICROORGANISMS FOLLOWING TRAUMA

Ann B. Bjornson, H. Stephen Bjornson, and Josef E. Fischer

Christ Hospital Institute of Medical Research

Cincinnati, Ohio 45219

SUMMARY

The temporal occurrence of multiple immunologic alterations resulting from thermal injury was investigated in guinea pigs with scald burns of 30% total body surface. Groups of 2-4 burned and sham-treated animals were sacrificed at 1-3 day intervals during 3 weeks postburn. C3 concentration, total and alternative complement pathway-mediated C3 fixation on Pseudomonas aeruginosa, proportion of native C3 to C3 cleavage fragments, and prostaglandin (PG) E_2 level were measured in serum or plasma. Bactericidal activity of peripheral polymorphonuclear leukocytes (PMN) against P. aeruginosa, proliferative responses of splenic lymphocytes to phytohemagglutinin and concanavalin A, and clearance of radiolabeled P. aeruginosa by the reticuloendothelial system were also determined. Complement consumption with concomitant reduction in C3 concentration and bacterial C3 fixation, and elevation of PGE_2 occurred within 3-6 h postburn. These alterations were accompanied by reduction in intrinsic PMN bactericidal activity, suppression of PMN bactericidal activity by serum, and a minor decrease in blood clearance of P. aeruginosa. Complement and PMN dysfunction returned to normal by the end of the first week postburn. A clear temporal separation in the occurrence of depression in lymphoproliferative responses was evident, since this alteration was not observed until 4 days postburn and was maximal during 7-9 days postburn. Our results support the concept that there is a continuum of immunologic alterations resulting from thermal injury and that arachidonate metabolites and complement cleavage fragments participate in its initiation.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (DHEW Publication No. (NIH) 78-23, revised 1978). Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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Introduction

Thermal injury is the greatest known external assault on the inflammatory and immune systems. Almost every parameter of immunologic function studied to date has been shown to be abnormal. The alterations include depression in circulating immunoglobulins, complement, and fibronectin, reduction in opsonic activity, dysfunction of polymorphonuclear leukocytes (PMN) and monocytes, decrease in cell-mediated immune responses, increase in arachidonate metabolism, depression in clearance of particulate material by the reticuloendothelial system (RES), and appearance of circulating factors that reduce the functions of phagocytes and lymphocytes [1-4]. Investigations in this area have been carried out primarily in humans, in whom therapeutic measures undoubtedly complicate interpretation of the results. The studies have been limited for the most part to single humoral or cellular systems or processes. As a result, interrelationships among the various alterations and the role of injury per se in their induction are unknown. Moreover, the alterations occurring initially that may trigger subsequent alterations and that may be amenable to immunotherapeutic or pharmacologic circumvention are unknown. Such knowledge would enable development of new approaches for preserving host resistance following thermal injury which would reduce infectious complications and mortality.

Our approach to this problem has been to develop a hypothesis of possible interrelationships among immunologic alterations based primarily on recognized interactions among humoral and cellular systems [5]. The theory proposes that there are two primary pathways involving series of alterations with multiple interconnections between them. The hypothetical initiators of the pathways are arachidonic acid metabolites and complement

cleavage products. As a first step in proving our theory, we define in this investigation temporal relationships among alterations in multiple humoral and cellular systems in the guinea pig model of thermal injury.

Methods

Animals. Male and female Hartley guinea pigs weighing 300-350 g were purchased from Murphy Breeding Laboratories, Inc., Plainfield, IN. The animals were housed in separate cages and adapted to the new environment for 4-7 days. The animals were fed guinea pig chow ad libitum and were not fasted before injury.

Experimental thermal injury. A modification of the method of Herndon et al. [6] was used. Animals were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg; Butler & Co., Columbus, OH). Weight was measured, and dorsal hair was shaved. Fifteen mL of lactated Ringer's solution (Abbott Laboratories, North Chicago, IL) was administered intraperitoneally. Scald burns were applied by placing the animals in a custom-made insulated mold that exposed a 60 cm^2 area on the dorsum equal to approximately 15% of the total body surface. The area was immersed in 99°C water for 13 s. The animals were given oxygen and rested for 15 to 30 min. A second dorsal burn was then applied so that the total burn size approximated 30% of the body surface. After administration of additional oxygen, the animals were placed on heating blankets to reduce heat loss and thereby minimize stress. A second dose of Ringer's solution (15 mL) was administered intraperitoneally at 1.5 h postburn. The animals were then replaced in their cages. Survival of burned animals during 21 days postburn was 74%. Autopsies on selected animals at 24 h postburn showed that full-thickness burns had been produced without damage to the visceral organs. Sham injury was effected by immersing animals in tepid water. All other

procedures were identical to those used with the burned animals except oxygen was not adminstered.

Experimental design. Groups of burned and sham-treated guinea pigs were sacrificed at 3 h and 6 h postburn and on days 1, 2, 3, 4, 7, 9, 11, 14, 16, 18, and 21 postburn. Each group consisted of 2-4 animals except where indicated in the results. Animals were weighed, and specimens described below were obtained. Four separate sets of animals were used for harvesting PMN, spleen cells, and blood products, and for measuring RES clearance.

Collection and handling of specimens. Animals were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg). Blood was drawn by cardiac puncture into plastic syringes. Blood was heparinized for preparation of PMN (50 units/ml of blood). Blood for complete blood counts and platelet counts was added to microtainer capillary whole blood collectors containing ethylenediaminetetraacetate (EDTA) (Becton Dickinson & Co., Rutherford, NJ). For preparation of plasma, blood was collected in 3 mL vacuum tubes containing EDTA (Monoject, Division of Sherwood Medical Industries Inc., St. Louis, MO) and further supplemented with 60 uL of 250 um sodium meclofenamate (Warner-Lambert Co., Ann Arbor, MI). For preparation of serum, blood was clotted in glass tubes at room temperature and then stored at 4°C for up to 4 h. The tubes were centrifuged at 800 g for 10 min at 4°C. Plasma and serum were removed and stored in small aliquots at -70°C. After thawing, these specimens were refrozen and thawed again only once more if at all. Spleens were removed aseptically, and single cell suspensions were prepared by gentle teasing with glass microscope slides into RPMI 1640 (M.A. Bioproducts, Walkerville, MD) containing 0.35% HEPES (Sigma Chemical Co., St. Louis, MO), 0.1% gentamicin (Schering Corp.,

Kenilworth, NJ), and 5% heat-inactivated fetal calf serum (KC Biological, Lenexa, Kansas); this medium will be referred to hereafter as RPMI medium. After collection of specimens, animals were euthanized by intraperitoneal injection of 0.3 mL of T-61 euthanasia solution (American Hoechst Corp., Somerville, NJ).

Complete blood counts and platelet counts. These determinations were performed in the Central Hematology Laboratory of The Christ Hospital, Cincinnati, OH, using an Ortho ELT-800 hematology analyzer (Ortho Diagnostic Systems Inc., Westwood, MA).

Complement Determinations. The presence of C3 cleavage products in plasma was detected by crossed immunoelectrophoresis [7] using antiserum to guinea pig C3. This reagent was prepared in goats by repeated subcutaneous injection of guinea pig C3 (Cordis Laboratories, Miami, FL) in Freund's complete adjuvant (Difco Laboratories, Detroit MI). Serum concentrations of C3 were determined by radial immunodiffusion [8] using the same antiserum and pooled normal guinea pig serum as the reference standard. The amount of C3 in the reference serum was determined using purified guinea pig C3 as the standard. This material was prepared by the method of Thomas and Tack [9] and was homogeneous as assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis [10] and double immunodiffusion using antisera directed against guinea pig C3 and whole guinea pig serum (Colorado Serum Co., Denver, CO). The purified C3 was highly active as determined by hemolytic titration; C3 hemolytic activity was measured using erythrocyte intermediate and isolated complement components from Cordis Laboratories [11].

Total and alternative complement pathway-mediated C3 fixation on Pseudomonas aeruginosa was measured using a modification of the radioassay

described previously [12]. A clinical isolate of Pseudomonas aeruginosa, strain Wk, was grown for 6 hr at 37°C with aeration in trypticase soy broth (BBL, Cockeysville, MD). The bacteria were washed and resuspended to 1.0 x 10^9 colony forming units (cfu)/mL in Hank's balanced salt solution (M.A. Bioproducts, Walkersville, MD). Radioiodination of purified guinea pig C3 was carried out in tubes coated with Iodo-Gen (Pierce Chemical Co., Rockford, IL) [12]. Conditions were the same as those described previously except 100 µg of protein and 300 µCi of sodium(^{125}I) (Amersham, Arlington Heights, IL) were used. Serum was supplemented with labeled C3 and centrifuged for 3 min at 4°C in a Beckman model B microfuge (Beckman Instruments, Palo Alto, CA). The amount of labeled material added to the serum was adjusted so that samples used for analysis contained approximately 2.0 x 10^5 cpm. For measurement of total C3 fixation, 50 µL of bacteria were added to 50 µL of serum. For determination of alternative pathway-mediated C3 fixation, reaction mixtures were further supplemented with 10 µL of a solution containing 0.1 M ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid and 0.1 M magnesium chloride (MgEGTA). Controls contained 10 µL of 0.1 M EDTA to block complement activation. Reaction mixtures and controls were set up in duplicate. After incubation for 15 min at 37°C, 75 µL samples were removed to microfuge tubes containing 300 µL of 20% sucrose in isotonic veronal buffered saline, pH 7.4. The tubes were centrifuged for 5 min at 4°C in the microfuge. The tip of each tube containing the cell pellet was cut with a razor blade, and the pellet and remaining tube and its contents were counted separately in a Packard 5230 autogamma scintillation spectrometer (Packard Instrument Co., Downers Grove, IL). The percent of binding was calculated by dividing the cpm in the bacterial pellets by the total cpm and multiplying by 100. Average

control values were subtracted from average experimental values. Micrograms of bound C3 were calculated by multiplying the percent of binding by the μg of C3 in the samples. Final results were expressed as C3 molecules bound per cfu and were calculated by the formula [(μg bound C3 x 10^{-6}/180,000) x (6.023×10^{23})]/3.75×10^{7}.

Polymorphonuclear leukocyte (PMN) bactericidal assay. PMN were prepared from heparinized blood by dextran sedimentation followed by centrifugation through Hypaque-Ficoll and hypotonic lysis of contaminating erythrocytes [13]. PMN were suspended to 1.0×10^{7} cells/mL in Hank's balanced salt solution containing 0.1% gelatin (Difco Laboratories) (HBG). Suspensions contained 78%-92% PMN.

The method of Pruzanski et al. [14] was used to measure the bactericidal activity of PMN. Log phase P. aeruginosa Wk grown as described above were suspended to 2.0×10^{8} cfu/mL in 0.01 M phosphate buffered saline, pH 7.4. One hundred μL of bacteria, 200 μL of PMN, and 100 μL of serum were mixed together, and 300 μL was layered on coverslips (22 x 40 mm; no. 1 thickness). The coverslips were incubated in a moist chamber for 30 min at 37°C in 5% CO_2 and then washed with warmed Hank's balanced salt solution. The coverslips were stained for 1 min with 0.015% acridine orange (Fisher Scientific Co., Fair Lawn, NJ) in Hank's balanced salt solution, washed with the latter solution, and mounted as previously described. Dead (red) and live (green) PMN-associated bacteria were counted using a Zeiss fluorescent microscope (Carl Zeiss, Inc., Thornwood, NY); 50 PMN were viewed. Percent killing was calculated by dividing the number of dead bacteria by the total bacteria and multiplying by 100. The percent of PMN with associated bacteria was determined by dividing the number of PMN with associated bacteria by 50 and multiplying by 100. The number of bacteria

per PMN was calculated by dividing the total number of PMN-associated bacteria by 50. Preliminary experiments carried out in the absence of serum showed that phagocytosis and killing under the conditions employed were not dependent on opsonins.

Measurement of lymphoproliferative responses to mitogens. Cell suspensions were washed twice and adjusted to 3.0×10^6 spleen cells/mL in RPMI medium. In some experiments, the cells were first applied to columns of nylon wool [15]. Scrubbed nylon fiber (3 denier, 3.81 cm, type 200; Fenwal Laboratories, Deerfield, IL) was soaked in 0.2 N hydrochloric acid for 5 min and then rinsed with distilled water. The wool was dried, fluffed, and packed to the 12 mL mark in 20 mL plastic syringes. After autoclaving, the columns were equilibrated with RPMI medium and packed to a flow rate of 1 drop per s. The columns were warmed at 37°C for 45 min in 5% CO_2. Spleens were teased in 3 mL of RPMI medium, applied to the column, followed by 2 mL of medium. The column was incubated for 60 min, and cells were eluted by addition of 45 mL of RPMI medium. Recovery was 6-10% of cells applied. Rosette formation with rabbit erythrocytes was carried out by the method of Sandberg et al. [16]. Viability of untreated and nylon wool passed cells by trypan blue dye exclusion was greater than 75%.

Two hundred µL aliquots of the cell suspensions were dispensed into Falcon microtest III tissue culture plates (Becton Dickinson Labware, Oxnard, CA). In some experiments, 5% or 10% serum (vol/vol) was added to the cell suspensions prior to addition to the plates. Fifty µL of RPMI medium containing 0.5 µg of purified phytohemagglutinin (PHA; Burroughs Wellcome Co., Greenville, NC), 1 µg of concanavalin A (Con A; Sigma Chemical Co., St. Louis, MO), or no mitogen was added, and the plates were incubated at 37°C for 48 h in 5% CO_2. Fifty µL of RPMI medium containing

0.2 μCi of ^{3}H thymidine (6.7 Ci/mmol; New England Nuclear, Boston, MA) was added, and the incubation was continued for 18 h. Cells were collected using a Mash II harvester (M.A. Bioproducts), and radioactivity was counted in a Beckman LS 7000 liquid scintillation counter (Beckman Instruments, Palo Alto, CA); standard methods were used. Determinations were performed in triplicate. Percent reduction in proliferative responses was calculated by the formula $[1 - (A^{+mitogen} - A^{-mitogen})/(B^{+mitogen} - B^{-mitogen})] \times 100$, where A and B were mean cpm of the responses being compared.

Viability was measured in duplicate in separate plates containing cells cultured without mitogen. Fluorescent microscopy of fluorescein diacetate-ethidium bromide stained cells was used to assess viability [17]. Fifty cells were counted, and percent viability was calculated.

Measurement of bacterial clearance by the reticuloendothelial system (RES). A modification of the method of Brown et al. was used [18]. P. aeruginosa Wk was prepared as described in preceding sections except broth contained 2 μCi/mL of L-(^{35}S)methionine (Amersham) and the bacteria were washed and resuspended to 2.5×10^{9} cfu/mL in 0.01 M phosphate buffered saline, pH 7.4. The bacteria were divided into small aliquots and frozen at -20°C. Animals were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg) and injected intravenously with 0.25 mL of thawed bacteria ($3-4 \times 10^{5}$ cpm) via the lateral foot vein of the hind paw or the medial vein of the fore paw. At exactly 2 min, heparinized blood was drawn by cardiac puncture. The animal was immediately sacrificed by intracardiac injection of 0.3 mL of T-61 euthanasia solution, and the entire liver, spleen, and right lung were removed. The organs and a 2 mL aliquot of blood were counted in the gamma counter. Total cpm in the blood and lungs were estimated; blood volume was assumed to be 7% of the body weight [18],

and lung cpm were doubled. Blood and organ cpm were divided by total cpm injected to determine the percent of bacteria sequestered. Blood clearance was calculated by subtracting the percent sequestered from 100. The 2 min time interval was selected for measurement of clearance, because approximately one-half of the bacteria were cleared from circulation at this time, division of bacteria had not occurred, and bacteria had not been destroyed and released from the organs. In addition, abnormal blood and organ clearance was detected in complement-depleted animals at 2 min; for complement depletion, animals were injected intravenously with 70 units of cobra venom factor (Cordis Laboratories) 24 h prior to measurement of clearance.

Measurement of prostaglandin (PG) E_2. PGE_2 was measured in plasma by radioimmunoassay using monospecific antiserum from the Institute Pasteur (Paris, France). ^3H-PGE_2 (165 Ci/mmole) was purchased from New England Nuclear, and PGE_2 standard was obtained from the Upjohn Co., Kalamazoo, MI. Dilutions of antiserum, radioligand, standard, and samples were prepared in 0.1 M potassium phosphate buffer, pH 7.4, containing 0.9% sodium chloride, and 0.1% gelatin. One hundred µL of antiserum, radioligand, and sample were incubated at 4°C overnight. Free radioligand was precipitated by addition of 1 mL of dextran-coated charcoal (0.4% Norit A charcoal, 0.025% 70,000 mol wt dextran in the phosphate buffer), and the charcoal was deposited by centrifugation at 1600 x g for 15 min at 4°C. The supernatant containing the bound radioligand was decanted and counted in a Minaxi 4000 liquid scintillation spectrometer (Packard Instrument Co.). All samples were assayed in triplicate by serial dilution with dilution factors of 1, 2, and 4 to detect possible cross-reactivity with unknown substances. A curve parallel to the standard was observed with all samples. Fifty percent displacement of the bound radioligand required 11 pg of PGE_2

standard. To verify the accuracy of the assay, 3 random plasma samples were mixed with 100 pg/mL of PGE_2. Analysis of these samples showed the expected increase.

Statistical methods. Significant differences between data were determined by analysis of variance [19]. Correlations were assessed by regression analysis [20].

Results

Changes in hematologic profile and weight following thermal injury. All hematologic measurements were elevated in the burned animals during the first 3-6 h postburn (Table I). No major differences were observed thereafter between leukocyte counts in burned and sham-treated animals. The platelet count dropped on the day after injury and then returned to normal or supranormal. The erythrocyte count, hemoglobin, and hematocrit were decreased during 2-4 days postburn.

Weight loss was evident in the burned animals by 2 days postburn (Fig. 1). Maximal weight loss occurred at 9 days postburn. The animals began to gain weight thereafter.

Effects of thermal injury on the complement system. iC3b was detected in plasma from the burned animals at 3 and 6 h postburn but not at other time intervals. C3 cleavage fragments were not observed in plasma from sham-treated animals. A representative electrophoretic pattern is shown in Fig. 2.

Complement activation occurring early after thermal injury was accompanied by reduction in the serum concentration of C3, total C3 fixation on P. aeruginosa, and alternative pathway-mediated C3 fixation on this bacterium (Table II). During the first 2 days postburn, these measurements were significantly decreased in the burned animals as compared with the

controls ($p = <0.01$). Measurements in the burned animals returned to normal by 7-9 days postburn. A secondary decrease in alternative pathway-mediated C3 fixation was observed during 9-16 days postburn, although values were within the range of the controls ($0.36-2.01 \times 10^4$ molecules per cfu). Significant correlations were found between C3 concentration, total C3 fixation, and alternative pathway-mediated C3 fixation upon analysis of data from the burned animals ($r = >0.7$, $p = <0.01$); computations were performed with mean values from all days postburn. These findings suggest that C3 consumption is a primary contributing factor in the depression in complement dependent opsonization that occurs following thermal injury.

Alterations in PMN phagocytic and bactericidal activities. PMN from burned and sham-treated animals harvested during days 1-21 were separately pooled and assayed with homologous serum and pooled normal guinea pig serum. Two homologous sera were analyzed with each set of cells, and the results were averaged. Pooled normal serum was tested in duplicate, and the results were also averaged.

Bactericidal activity was significantly reduced during 9 days postburn upon testing with pooled normal serum or homologous serum ($p = <0.005$; Table III). The percent of PMN with associated bacteria and the number of bacteria per PMN, measurements reflecting adherence and phagocytosis, were not decreased substantively except on the first day postburn when cells were assayed with homologous serum.

The effects of sera from the burned animals and controls on the phagocytic and bactericidal activities of normal PMN were also investigated. Sera from the burned animals obtained during 9 days postburn significantly inhibited PMN bactericidal activity ($p = <0.001$; Fig. 3). The percent of PMN with associated bacteria was 100% in all determinations. The mean number of bacteria per PMN $\pm$ SEM was 5.27 ± 0.16 and 5.16 ± 0.10 in the

presence of sera from the burned animals and controls respectively (n = 26 for each group); fluctuation in results throughout the 21 day period was not observed. These results and those described above suggest that (1) reduction in PMN bactericidal activity is not reversed by incubation with normal serum, (2) inhibitory serum factors contribute to this alteration, and (3) early after thermal injury, these factors depress phagocytosis in addition to bactericidal activity.

Burn-induced changes in lymphoproliferative responses to mitogens. Spleen cell suspensions were initially cultured with mitogens in the absence of serum. Results from each group were averaged, and these averages were used to calculate the percent reduction in the proliferative response of spleen cells from the burned animals relative to the controls. Responses to both mitogens were reduced in the burned animals beginning at 4 days postburn and continuing through 11 days postburn (Fig. 4). Responses returned to normal on day 14 and then became abnormal again on days 16 and 18 postburn. Viability in all cultures was greater than 70%, indicating that the reduced responses in the burned animals were not related to cell death.

Sera from experimental and control groups were tested for their effects on the proliferative responses of normal spleen cells to PHA and Con A. Responses were compared with those observed in the absence of serum. Certain sera from both groups inhibited the responses when tested at a concentration of 5%, and the greatest effect was observed using Con A as the mitogen (Table IV). Differences between inhibitory effects of experimental and control sera were less than 20%. While the demonstration of inhibitory activity was quite variable with respect to the test sera and mitogen, it was a reproducible finding unrelated to the extent of the response. Sera obtained at 3 and 6 h and on days 9, 11, 14, 16, and 18

were retested at a concentration of 10%. Inhibitory effects of the sera increased using both mitogens, however differences in the inhibitory effects of experimental and control sera were no greater than those observed upon testing of 5% sera (results not shown). Viability in all experiments was greater than 70%. These results fail to document a suppressive effect of sera related to thermal injury.

Proliferative responses of T cell-enriched and unenriched spleen cells to the mitogens were also compared. Cells were harvested on days 4, 7, 9, and 11 postburn. For enrichment of T cells, spleen cells were passed over nylon wool columns. Rosette formation was 26-32% with untreated cells and 58-67% with nylon wool passed cells, confirming that nylon wool passage enriched T cells. Results at each time interval were averaged, and those from the burned animals were analysed relative to those from the control animals using both cell preparations. The degree of hyporesponsiveness observed with nylon wool passed cells from the burned animals was equal to or greater than that observed with untreated cells (Table V). These results suggest that reduction in proliferative responses following thermal injury is related at least in part to an alteration in T cell function.

RES clearance of radiolabeled bacteria following injury. Reduction in blood clearance of P. aeruginosa was observed in the burned animals on the first day postburn (Table VI). This change was accompanied by an increase in sequestration of bacteria in the lungs. However, results were not statistically different from control data. Bacterial sequestration in the liver and spleen was equivalent in the burned animals and controls during the entire period of observation.

Effects of thermal injury on arachidonate metabolism. PGE_2 was significantly elevated in plasma from the burned animals as compared with the

controls ($p = 0.0015$; Fig. 5). The highest levels were observed during the first week postburn. There was considerable variability in some of the results from burned animals sacrificed on the same postburn day. The reason for this finding is not known, but it does not appear to be related to interfering factors. Determinations on additional animals are currently in progress.

Discussion

The guinea pig model of thermal injury used in our investigation has been well characterized. Developmental studies by Herndon et al. [6] showed that the metabolic response of guinea pigs to thermal injury simulates the response occurring in humans. The temporal sequence of burn wound colonization with bacteria is also similar, although the species of bacteria are somewhat different [21]. Hematologic changes mimic those observed in the human except for the lack of leukopenia which is probably compensated for by splenic hematopoiesis in the guinea pig and other rodents [22]. The primary reason for selecting the guinea pig as opposed to other well characterized rodent models is that its immune system is "corticosteroid resistant" like the human and therefore immunologic changes induced by endogenous glucocorticoids should be similar in these species [23]. Using the guinea pig model, we established the chronology of key changes in the inflammatory and immune systems that result from moderately severe burns.

The first line of host defense, comprised of PMN and serum opsonins, was profoundly altered early after thermal injury. Within 24 h postburn, complement consumption had begun, and both total and alternative complement pathway-mediated opsonization of P. aeruginosa were reduced. In addition, the intrinsic bactericidal activity of PMN was depressed, and factors were

present in serum that inhibited PMN bactericidal activity. All of these alterations normalized by the end of the first week postburn. A secondary reduction in alternative pathway-mediated opsonization was observed during the second week. This change may reflect continued complement consumption or the presence of inhibitory factors. Inhibition of the alternative pathway has been observed during the same time interval in burned humans [24-26].

Since the early phase of complement consumption coincided temporally with PMN dysfunction, it is likely that complement cleavage fragments play a role in down-regulating PMN function. Fragments, such as C5a, probably saturate PMN receptors resulting in aggregation and degranulation, hallmarks of the phenomenon known as "non-specific deactivation" [27-29]. The demonstration of serum-mediated inhibition of PMN antibacterial function may however be related to other factors, since this phenomenon continued to be observed after complement fragments were detected in circulation. It should be noted that the method used to detect these fragments, crossed immunoelectrophoresis, is not highly sensitive. More sensitive techniques might have enabled demonstration of circulating complement fragments in temporal association with serum-mediated inhibition of PMN function.

Despite the marked reduction in opsonization and PMN bactericidal activity against P. aeruginosa occurring early after injury, only a minor reduction in blood clearance of this bacterium was observed. Bacterial sequestration by the fixed macrophages of the lung, spleen, and liver was not adversely affected by thermal injury. The elevation in numbers of peripheral phagocytes occurring during this period may aid in protection against systemic P. aeruginosa challenge.

Unlike the alterations in complement and PMN function that appeared within several hours of the trauma, lymphoproliferative responses to T cell mitogens did not become depressed until 2-4 days postburn. These responses were maximally reduced at 7-9 days postburn when the alterations in complement and PMN had resolved. The clear temporal separation in results suggests that mediators of these alterations are distinct and that the reduction in lymphoproliferative responses is a secondary sequela of injury. It may occur as a consequence of nutritional depletion, since weight loss in the burned animals occurred during the same interval. In this regard, there is a well recognized association between malnutrition and reduction in cell-mediated immune responses [30-33].

No role could be found for suppressive serum factors in the depression of lymphoproliferative responses. Sera from some of the burned animals inhibited responses of normal spleen cells, however the same extent of inhibition was observed with certain control sera. The methods used in our investigation were similar to those used in previous studies in which a marked suppressive effect of sera from burned subjects relative to controls was noted [34-36]. The occurrence of suppressive serum factors in thermally injured humans may be related to systemic infection or drugs that were not present in our animal model. In this regard, iodine absorbed as a result of betadine treatment has been implicated as a suppressive serum factor [37] and so has bacterial endotoxin [38]. These factors and others undoubtedly increase immunosuppression mediated by cells.

Our results suggest that T cells are involved in the immunosuppression associated with thermal injury, however other cells such as macrophages probably also have suppressive effects or reduced function. Preliminary data from our laboratory obtained with inbred strain 2 guinea pigs support

the idea that dysfunction of lymphoid cells and excess suppressor function both contribute to the depression in the immune system occurring after thermal injury [unpublished observations]. Previous studies by other investigators in mice and humans have demonstrated a role for suppressor T cells [36,39,40], suppressor macrophages [41,42], and decreased numbers of helper T cells [43,44] in the alteration of immunocompetence associated with thermal injury.

In agreement with other reports documenting an increase in arachidonate metabolism following thermal injury [45-47], we observed elevated plasma levels of PGE_2 in the burned animals during the 21 day period of observation. It is probable that PGE_2 and other products of arachidonate metabolism play a major role in suppression of host defenses accompanying thermal injury, and this role warrants further study. In our investigation, the greatest increase in PGE_2 levels occurred during the first week postburn. Since PG of the E series are known to inhibit release of lysosomal enzymes, superoxide production, and chemotaxis of PMN [48-51], they may represent the serum factors that inhibit PMN bactericidal activity. PGE_2 has also been shown to suppress lymphoproliferative responses but at lower concentrations than those detected in plasma from the burned animals [52]. This is presumably why sera from these animals did not suppress mitogenic responses to a greater extent than control sera.

Several parts of our original hypothesis [5] concerning interrelationships among immunologic alterations resulting from thermal injury can now be ruled out. Firstly, the concept that complement cleavage fragments in circulation contribute to depression in lymphoproliferative responses is probably incorrect, since a temporal association between these variables was not observed. Secondly, it was postulated that reduction in

lymphoproliferative responses might be accompanied by local secretion and entry into circulation of "nonantigen-specific suppressor factors" that further reduce lymphoproliferative responses and possibly also other responses. No evidence for this theory was obtained in the present study, since burn-related serum suppression of lymphoproliferative responses was not observed.

Support was gained for the idea previously put forth that defineable pathways involving series of burn-induced immunologic alterations exist. As postulated, complement consumption and increase in arachidonate metabolism were early events whose occurrence was temporally associated with alterations in antibacterial properties of PMN and immune function. Selective blockade of these early events will be the next step in determining if they are pivotal in the continuum of immunologic alterations and if this approach has therapeutic benefit.

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References

1. Miller, S.E., C.L. Miller, and D.D. Trunkey. 1982. The immune consequences of trauma. Surg. Clin. N. Am. 62:167-181.

2. Ninnemann, J.L. 1982. Immunologic defenses against infection: alterations following thermal injury. J. Burn Care Rehab. 3:355-398.

3. Munster, A.M. 1984. Immunologic response of trauma and burns. Am. J. Med. 76(3A):142-145.

4. Gelfand, J.A. 1984. Infections in burn patients: a paradigm for cutaneous infection in the patient at risk. Am. J. Med. 76(5A):158-165.

5. Bjornson, A.B., and H.S. Bjornson. 1984. Theoretical interrelationships among immunologic and hematologic sequelae of thermal injury. Rev. Inf. Dis. 6:704-714.

6. Herndon, D.N., D.W. Wilmore, and A.D. Mason, Jr. 1978. Development and analysis of a small animal model simulating the human postburn hypermetabolic response. J. Surg. Res. 25:394-403.

7. Weeke, B. 1973. Crossed electrophoresis. Scand. J. Immunol. 2(Suppl. 1):47-56.

8. Mancini, G., A.O. Carbonara, and J.F. Heremans. 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2:235-254, 1965.

9. Thomas, M.L., and B.F. Tack. 1983. Identification and alignment of a thiol ester site in the third component of guinea pig complement. Biochemistry 22:942-947.

10. Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.

11. Vroon, D.H., D.R. Schultz, and R.M. Zarco. 1970. The separation of nine components and two inactivators of components of complement in human serum. Immunochemistry 7:43-61.

12. Leist-Welsh, P., and A.B. Bjornson. 1982. Immunoglobulin-independent utilization of the classical complement pathway in opsonophagocytosis of Escherichia coli by human peripheral leukocytes. J. Immunol. 128:2643-2651.

13. McCarthy, J.P., R.S. Bodroghy, P.B. Jahrling, and P.Z. Sobocinski. 1980. Differential alterations in host peripheral polymorphonuclear leukocyte chemiluminescence during the course of bacterial and viral infections. Infect. Immun. 30:824-831.

14. Pruzanski, W., S. Saito, and D.W. Nitzan. 1983. The influence of lysostaphin on phagocytosis, intracellular bactericidal activity, and chemotaxis of human polymorphonuclear cells. J. Lab. Clin. Med. 102:298-305.

15. Julius, M.H., E. Simpson, and L.A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. Eur. J. Immunol. 3:645-649.

16. Sandberg, G., and O. Soder. 1984. Studies on thymocyte subpopulations in guinea pigs. V. Rosette formation as a tool to separate thymocyte growth factor responsive and mitogen reactive cells. Int. J. Arch. Allergy Appl. Immunol. 74:297-304.

17. Takasugi, M. 1971. An improved fluorochromatic cytotoxic test. Transplantation 12:148-151.

18. Brown, E.J., S.W. Hosea, C.H. Hammer, C.G. Burch, and M.M. Frank. 1982. A quantitative analysis of the interactions of antipneumococcal antibody and complement in experimental pneumococcal bacteremia. J. Clin. Invest. 69:85-98.

19. Remington, R.D., and M.A. Schork. 1970. In Statistics with Applications to the Biological and Health Sciences. Prentice-Hall, Inc., Englewood Cliffs, NJ, 282-288.

20. Remington, R.D., and M.A. Schork. 1970. In Statistics with Applications to the Biological and Health Sciences. Prentice-Hall, Inc., Englewood Cliffs, NJ, 273-275.

21. Bjornson, A.B., H.S. Bjornson, N.A. Lincoln, and W.A. Altemeier. 1984. Relative roles of burn injury, wound colonization, and wound infection in induction of alterations of complement function in a guinea pig model of burn injury. J. Trauma 24:106-115.

22. Wallner, S., R. Vautrin, J. Murphy, S. Anderson, and V. Peterson. 1984. The haematopoietic response to burning: studies in an animal model. Burns 10:236-251.

23. Parillo, J.E., and A.S. Fauci. 1979. Mechanisms of glucocorticoid action on immune processes. Ann. Rev. Pharmacol. Toxicol. 19:179-201.

24. Bjornson, A.B., W.A. Altemeier, and H.S. Bjornson. 1976. Reduction in C3 conversion in patients with severe thermal injury. J. Trauma 16:905-911.

25. Bjornson, A.B., W.A. Altemeier, and H.S. Bjornson. 1977. Changes in humoral components of host defense following burn trauma. Ann. Surg. 186:88-96.

26. Bjornson, A.B., H.S. Bjornson, and W.A. Altemeier. 1981. Reduction in alternative complement pathway mediated C3 conversion following burn injury. Ann. Surg. 194:224-231.

27. Ward, P.A., and E.L. Becker. 1968. The deactivation of rabbit neutrophils by chemotactic factor and the nature of the activatable esterase. J. Exp. Med. 127:693-709.

28. Nelson, R.D., R.T. McCormack, V.D. Fiegel, and R.L. Simmons. 1978. Chemotactic deactivation of human neutrophils: evidence for nonspecific and specific components. Infect. Immun. 22:441-444.

29. Solomkin, J.S., L.A. Cotta, J.K. Brodt, J.W. Hurst, and C.K. Ogle. 1984. Neutrophil dysfunction in sepsis. III. Degranulation as a mechanism for nonspecific deactivation. J. Surg. Res. 36:407-412.

30. Good, R.A. 1981. Nutrition and immunity. J. Clin. Immunol. 1:3-11.

31. Keusch, G.T. 1982. Nutrition and infections. Compr. Ther. 8:7-15.

32. Santos, J.I., J.L. Arredondo, and J.J. Vitale. 1983. Nutrition, infection and immunity. Pediatr. Ann. 12:182-194.

33. Chandra, R.K., and S. Tejpar. 1983. Diet and immunocompetence. Int. J. Immunopharmacol. 5:175-180.

34. Wolfe, J.H.N., I. Saporoschetz, A.E. Young, N.E. O'Connor, and J.A. Mannick. 1981. Suppressive serum, suppressor lymphocytes, and death from burns. Ann. Surg. 193:513-520.

35. Wolfe, J.H.N., A.V.O. Wu, N.E. O'Connor, I. Saporoschetz, and J.A. Mannick. 1982. Anergy, immunosuppressive serum, and impaired lymphocyte blastogenesis in burn patients. Arch. Surg. 117:1266-1271.

36. McIrvine, A.J., J.B. O'Mahony, I. Saporoschetz, and J.A. Mannick. 1982. Depressed immune response in burn patients. Use of monoclonal antibodies and functional assays to define the role of suppressor cells. Ann. Surg. 196:297-304.

37. Ninnemann, J.L., and M.D. Stein. 1981. Suppressor cell induction by povidone-iodine: in vitro demonstration of a consequence of clinical burn treatment with betadine. J. Immunol. 126:1905-1908.

38. Ninnemann, J.L., J.T. Condie, S.E. Davis, and R.A. Crockett. 1982. Isolation of immunosuppressive serum components following thermal injury. J. Trauma 22:837-844.

39. Miller, C.L., and B.J. Claudy. 1979. Suppressor T-cell activity induced as a result of thermal injury. Cell. Immunol. 44:201-208.

40. Ninnemann, J.L. 1980. Immunosuppression following thermal injury through B cell activation of suppressor T cells. J. Trauma 20:206-213.

41. Suzuki, F., and R.B. Pollard. 1982. Mechanism for the suppression of gamma-interferon responsiveness in mice after thermal injury. J. Immunol. 129:1811-1815.

42. Hansbrough, J.F., V. Peterson, E. Kortz, and J. Piacentine. 1983. Postburn immunosuppression in an animal model: Monocyte dysfunction induced by burned tissue. Surgery 93:415-423.

43. Antonacci, A.C., R.A. Good, and S. Gupta. 1982. T-cell subpopulations following thermal injury. Surg. Gynecol. Obstet. 155:1-8.

44. Antonacci, A.C., S.E. Calvano, L.E. Reaves, A. Prajapati, R. Bockman, K. Welte, R. Mertelsmann, S. Gupta, R.A. Good, and G.T. Shires. 1984. Autologous and allogeneic mixed-lymphocyte responses following thermal injury in man: the immunomodulatory effects of interleukin 1, interleukin 2, and a prostaglandin inhibitor, WY-18251. Clin. Immunol. Immunopathol. 30:304-220.

45. Heggers, J.P., G.L. Loy, M.C. Robson, and E.J. Del Beccaro. 1980. Histological demonstration of prostaglandins and thromboxanes in burned tissue. J. Surg. Res. 28:110-117.

46. Harms, B.A., B.I. Bodai, M. Smith, R. Gunther, J. Flynn, and R.H. Demling. 1981. Prostaglandin release and altered microvascular integrity after burn injury. J. Surg. Res. 31:274-280.

47. Shires, G.T., and P. Dineen. 1982. Sepsis following burns, trauma, and intra-abdominal infections. Arch. Int. Med. 142:2012-2022.

48. Zurrier, R.B., G. Weissmann, S. Hoffstein, S. Kammerman, and H.H. Tai. 1974. Mechanisms of lysosomal enzyme release from human leukocytes. II. Effects of cAMP and cGMP, autonomic antagonists, and agents which affect microtubule function. J. Clin. Invest. 53:297-309.

49. Rivkin, I., J. Rosenblatt, and E.L. Becker. 1975. The role of cyclic AMP in the chemotactic responsiveness and spontaneous motility of rabbit peritoneal neutrophils. The inhibition of neutrophil movement and the elevation of cyclic AMP levels by catecholamines, prostaglandins, theophylline and cholera toxin. J. Immunol. 115:1126-1134.

50. Fantone, J.C., and D.A. Kinnes. 1983. Prostaglandin E_1 and prostaglandin I_2 modulation of superoxide production by human neutrophils. Biochem. Biophys. Res. Commun. 113:506-512.

51. Fantone, J.C., W.A. Marasco, L.J. Elgas, and P.A. Ward. 1983. Anti-inflammatory effects of prostaglandin E_1: in vivo modulation of the formyl peptide chemotactic receptor on the rat neutrophil. J. Immunol. 130:1495-1497.

52. Goodwin, J.S., and J. Ceuppens. 1983. Regulation of the immune response by prostaglandins. J. Clin. Immunol. 3:295-315.

ABBREVIATIONS

cfu - colony forming units

Con A - concanavalin A

EDTA - ethylenediaminetetraacetate

HBG - Hank's balanced salt solution containing 0.1% gelatin

MgEGTA - 0.1 M ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid and 0.1 M magnesium chloride

PG - prostaglandin

PHA - phytohemagglutinin

PMN - polymorphonuclear leukocytes

RES - reticuloendothelial system

RPMI medium - RPMI 1640 containing 0.35% HEPES, 0.1% gentamicin, and 5% heat-inactivated fetal calf serum

Table I. Changes in Complete Blood Count and Platelet Count Following Thermal Injury

Days Postburn	N*	Platelets x 10^3	Leukocytes x 10^3	Erythrocytes x 10^3	Hemoglobin g	Hematocrit %
3 h	2	636 ± 97	15.2 ± 4.2	5.79 ± 0.1	15.0 ± 0.1	45.1 ± 0.1
6 h	2	383‡	12.4 ± 4.4	5.30 ± 0.1	14.1 ± 0.2	43.8 ± 0.6
1	3	299 ± 16	6.2 ± 3.9	4.42 ± 0.9	12.2 ± 2.4	38.8 ± 8.7
2	3	332 ± 47	3.5 ± 1.0	4.01 ± 0.5	10.3 ± 1.0	33.4 ± 3.8
3	2	529 ± 96	4.5 ± 0.9	4.20 ± 0.1	11.0 ± 0.3	35.4 ± 1.3
4	2	517 ± 27	3.3 ± 1.2	3.85 ± 0.4	10.1 ± 1.0	33.4 ± 3.2
7	2	698 ± 96	4.1 ± 0.3	4.49 ± 0.1	12.1 ± 0.1	38.8 ± 0.6
9	2	752 ± 32	3.5 ± 0.1	4.50 ± 0.2	11.1 ± 1.1	36.4 ± 3.1
11	2	465 ± 180	4.2 ± 0.9	4.89 ± 0.1	12.0 ± 0.4	39.8 ± 0.8
14	2	825 ± 58	3.1 ± 0.9	4.78 ± 0.1	12.3 ± 0.1	40.8 ± 0.7
16	2	650 ± 142	3.7 ± 0.1	4.71 ± 0.1	12.5 ± 0.3	41.0 ± 0.7
18	1	482	2.8	5.45	15.1	48.2
21	1	303	5.7	4.84	12.0	40.9
Controls	24	457 ± 19	4.8 ± 0.4	4.74 ± 0.1	12.5 ± 0.1	40.5 ± 0.5

Data are presented as mean ± SEM. Measurements on days 18 and 21 were from one animal in both the experimental and control groups.

*Number of animals.

‡Single determination.

Table II. Effects of Thermal Injury on Serum C3 Concentration and C3 Fixation on P. aeruginosa

Days Postburn	N*	C3 µg/mL	C3 Molecules Bound Per cfu ($\times 10^{-4}$)	
			Total	Alternative Pathway
3 h	2	862 ± 238	0.94 ± 0.07	0.31 ± 0.23
6 h	2	885 ± 45	0.80 ± 0.17	0.20 ± 0.13
1	2	890 ± 60	0.65 ± 0.01	0.43 ± 0.08
2	3	817 ± 73	1.03 ± 0.03	0.46 ± 0.16
3	2	1475 ± 40	1.62 ± 0.12	0.99 ± 0.16
4	2	1162 ± 78	1.25 ± 0.09	0.34 ± 0.22
7	2	1555 ± 35	1.52 ± 0.10	0.70 ± 0.02
9	2	1520 ± 0	2.06 ± 0.12	0.51 ± 0.03
11	2	1270 ± 70	1.51 ± 0.38	0.41 ± 0.11
14	2	2070 ± 130	2.33 ± 0.61	0.63 ± 0.08
16	2	1605 ± 115	1.71 ± 0.01	0.43 ± 0.04
18	3	2230 ± 170	3.83 ± 0.29	1.01 ± 0.17
21	2	2330 ± 170	4.81 ± 1.13	0.76 ± 0.58
Controls	24	1300 ± 40	2.25 ± 0.14	0.86 ± 0.09

Data are presented as mean ± SEM. Controls were not analyzed on day 21.

*Number of animals.

Table III. PMN Bactericidal Activity in the Presence of Homologous Serum and Pooled Normal Guinea Pig Serum

	PMN with Associated Bacteria (%)		No. Bacteria Per PMN		Killing (%)	
Days Postburn	Homologous Serum	Pooled Normal Serum	Homologous Serum	Pooled Normal Serum	Homologous Serum	Pooled Normal Serum
1	87	100	3.7	5.2	59	45
2	95	100	5.2	6.9	47	64
3	100	100	4.1	5.3	58	62
4	100	100	5.9	4.6	61	66
7	100	100	4.9	4.7	59	66
9	100	100	4.4	4.2	70	72
11	100	100	4.7	4.5	76	75
14	100	100	4.9	5.4	75	79
16	100	100	5.5	5.5	76	78
18	100	100	5.3	5.2	76	79
21	100	100	5.7	5.7	77	80
Controls	100 $\pm$ 0	100 $\pm$ 0	5.24 $\pm$ 0.22	5.17 $\pm$ 0.19	77 $\pm$ 0.6	80 $\pm$ 0.4

Experimental data are presented as averages of 2 determinations. Control data are presented as mean $\pm$ SEM of 11 determinations.

Table IV. Effect of Sera from Burned and Sham-Treated Guinea Pigs on the Proliferative Response of Normal Spleen Cells to PHA and Con A

	Reduction in Response (%)					
	PHA			Con A		
Days Postburn	Experimental	Control	Experimental -Control	Experimental	Control	Experimental -Control
3 h	0	0	0	24	8	16
6 h	0	0	0	47	43	4
1	8	30	-22	39	40	-1
2	13	15	-2	37	46	-9
3	0	0	0	8	7	1
4	0	0	0	0	0	0
7	0	0	0	0	0	0
9	0	3	-3	17	7	10
11	10	0	10	62	53	9
14	0	0	0	55	48	7
16	38	32	6	29	13	16
18	0	0	0	33	21	12
21	0	0	0	33	51	-18

Sera were tested at a concentration of 5% (vol/vol). Responses without serum in mean $\pm$ 1SD were 43,324 $\pm$ 21,185 for PHA and 57,022 $\pm$ 23,657 for Con A.

Table V. Reduction in the Proliferative Responses of Nylon Wool Passed Spleen Cells from Burned Guinea Pigs

	Reduction in Response (%)			
	PHA		Con A	
Days Postburn	Nylon Wool Passed	Untreated	Nylon Wool Passed	Untreated
4	35	26	73	20
7	21	35	38	46
9	39	42	60	53
11	80	77	61	24

Control responses in mean ± 1SD were 59,885 ± 7,166 cpm (nylon wool passed cells, PHA), 54,808 ± 11,231 cpm (untreated cells, PHA), 43,762 ± 5,448 cpm (nylon wool passed cells, Con A), and 50,642 ± 10,696 cpm (untreated cells, Con A).

Table VI. Blood Clearance and Organ Sequestration of P. aeruginosa following Thermal Injury

Days Postburn	N*	Blood Clearance (%)	Organ Sequestration (%)		
			Spleen	Lung	Liver
3 h	4	62 ± 6	1.5 ± 0.3	11 ± 2	33 ± 10
6 h	4	46 ± 14	2.6 ± 1.1	10 ± 2	27 ± 4
1	4	40 ± 5	1.6 ± 0.3	16 ± 3	34 ± 6
2	2	52 ± 2	1.7 ± 0.2	15 ± 2	33 ± 1
3	2	43 ± 4	1.9 ± 0.3	15 ± 4	35 ± 1
4	2	59 ± 1	1.6 ± 0.2	14 ± 2	40 ± 1
7	2	54 ± 8	1.5 ± 0.1	12 ± 3	36 ± 5
9	2	52 ± 13	1.8 ± 0.7	15 ± 4	33 ± 6
12	2	69 ± 2	1.2 ± 0.2	8 ± 1	43 ± 1
14	2	50 ± 2	2.2 ± 0.2	13 ± 2	30 ± 4
16	2	64 ± 10	3.7 ± 0	8 ± 1	33 ± 7
18	3	53 ± 5	2.3 ± 0.6	13 ± 3	29 ± 3
21	2	58 ± 2	2.5 ± 0.3	11 ± 1	35 ± 3
Controls	33	52 ± 2	2.4 ± 0.2	10 ± 1	36 ± 1

Data are presented as mean ± SEM.

*Number of animals.

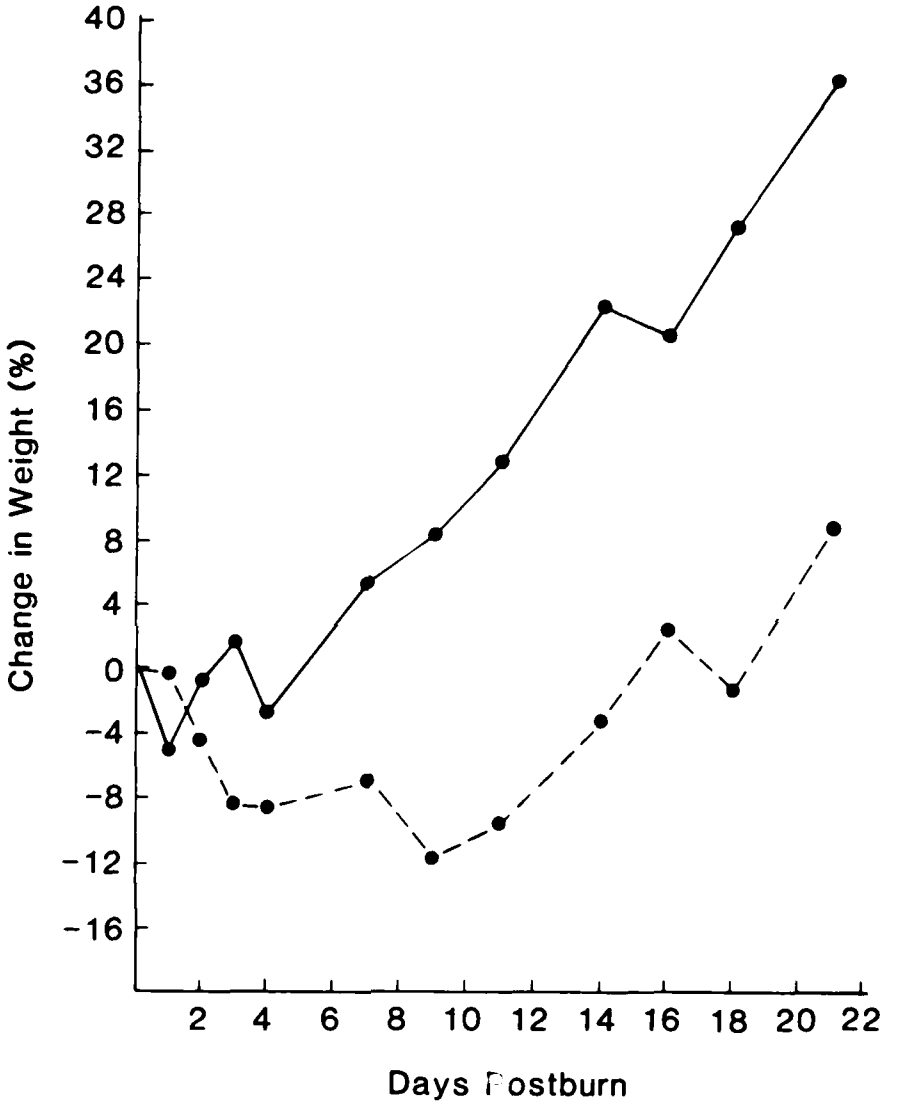

Figure 1. Change in weight in burned (---) and sham-treated (——) guinea pigs during 21 days of observation. Data were derived from a total of 176 animals. Points at each time interval are means from 4-16 animals.

Figure 2. Crossed immunoelectrophoretic analysis of plasma from burned (top) and sham-treated (bottom) guinea pigs. The plasma was obtained at 6 h after injury. The anode was at the right.

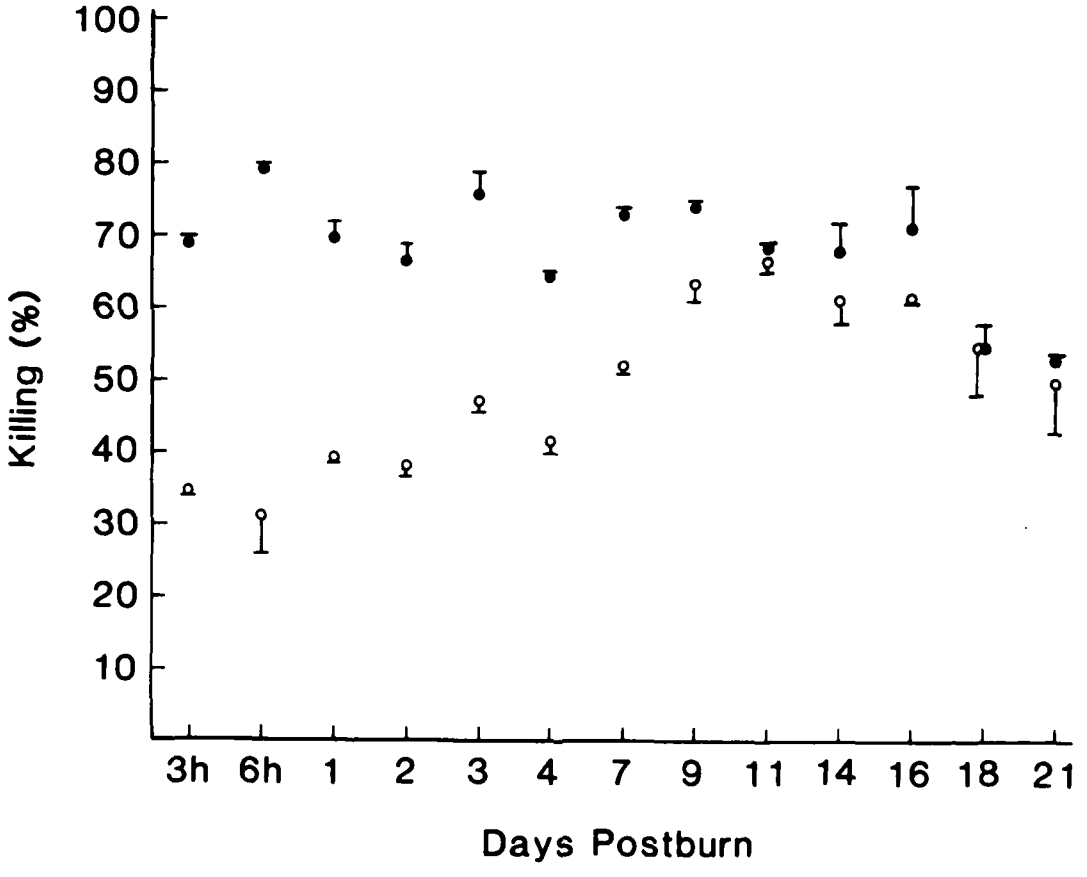

Figure 3. Effect of sera from burned (O) and sham-treated (●) guinea pigs on the bactericidal activity of normal PMN. The mean and SEM of 2 determinations are shown.

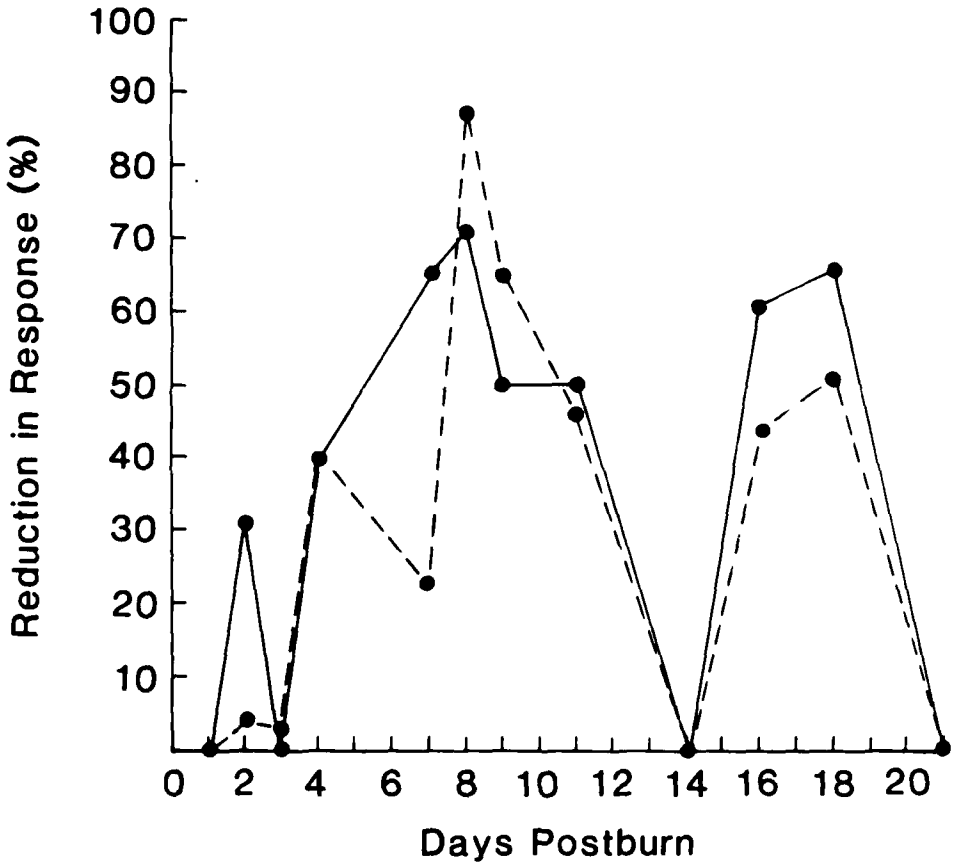

Figure 4. Reduction in the proliferative responses of spleen cells to T cell mitogens during 21 days postburn. The solid and dotted lines show data with PHA and Con A respectively. Control responses in mean ± 1SD were 37,049 ± 14,206 cpm for PHA and 46,295 ± 13,752 cpm for Con A.

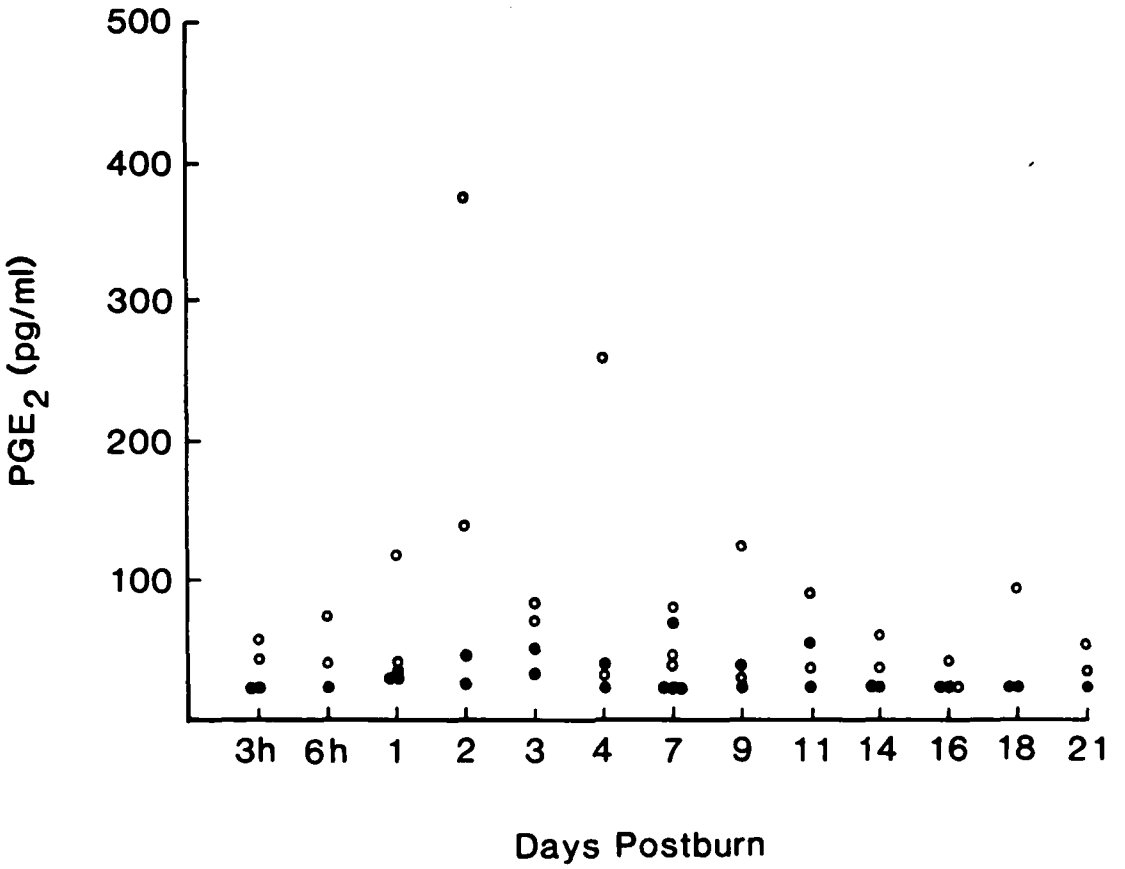

Figure 5. Levels of PGE_2 in plasma from burned (O) and sham-treated (●) guinea pigs during 21 days postburn.

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