A Systematic Study of Host Defense Processes in Badly Injured Patients

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A prospective study of factors predisposing to infection in badly injured patients has disclosed: (1) the dominant roles of two specific parameters: monocyte antigen presenting capacity, and opsonic capacity of diluted serum; (2) the potential value of further assessment of: the predictive value of plots of activated T-cells/total T-cells versus monocyte antigen presenting capacity, the apparent protective effect of the ability to sharply increase specific IgM in response to infection, and the apparent protective effect of cytomegalovirus (CMV) infection in the first 28 days after injury against major bacterial infection; (3) the lack of value of analysis of other T- and B-cell subsets in such patients; and (4) the need to clarify CMV and transfusion status with respect to interpretation of such data. The specific role of variable transfusion and of specific serum immunoglobulins will require further and more discriminating study.

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THE CONTINUING BATTLE among the surgeon, his patient, and the microbial environment in which both reside has tantalizingly offered solution and victory for more than 100 years. It is quite clear that the impact of surgical asepsis and judicious prophylactic and therapeutic antibiotic use has ameliorated the process in favor of the surgeon and his patient. Indeed, as the technical skills of surgery have proceeded apace, surgeons are conducting ever more extensive operative procedures on increasingly debilitated patients. The net balance is one of major progress that has, to some extent, been concealed because of a shifting battlefront. There continue to be examples where overwhelming bacterial contamination defeats even the most judicious and skillful surgeon and his normal patient. Increasingly, surgeons have been deeply concerned with the other side of that coin, that is, the patient who falls prey to an infection for no obvious or sufficient reason. In other words, the patient is surgically well cared for, his illness and/or injuries appropriately treated, antibiotics have been wisely used, and supportive care has been ideal; even now under some sets of circumstances, invasive infection contributes to tissue destruction and delayed recovery or even death.

Over the last 10 years, numerous surgical investigators have assayed one or more components of the host–pathogen interaction, and much has been learned regarding the value and limitations of antibiotics as well as the double-edged sword of the therapeutic foreign body. Many of these inquiries have involved unidimensional examinations of putative host defenses in both the clinical and laboratory setting.

It is the purpose of this report to describe broadly based inquiries into multiple aspects of the host defense process as reflected by the ongoing and repetitive studies of high-risk, badly injured patients cared for in a single university hospital setting. It was anticipated that such a set of inquiries might disclose sophisticated interactions and clarify, once and for all, which, if any, of these specific and nonspecific immunologic factors contribute to the propensity for infection on the part of some surgical patients. Intrinsic to such an inquiry is the definition of normal recovery.

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Patients and Methods

Patient Population

Twenty consecutive patients admitted under the care of the emergency surgical services at the Humana Hospital University qualified for study as specified below. Only patients who had sustained major trauma resulting in an Injury Severity Score[1] of at least 20 were accepted. Patients with a previous medical history of myocardial infarction, organ failure, neoplastic disease, or current immunosuppressive therapy (steroids or cytotoxic drugs) were excluded. Patients transferred from other centers after initial stabilization were accepted; however, those transferred after prolonged care were excluded. Informed consent was obtained from the patient or relatives in all cases.

Details of the patients studied are given in Table 1. To clarify the effect of blood transfusion on response to trauma, patients were further classified according to the volume of blood transfusion received within 48 hours of admission. Patients receiving fewer than 10 units were arbitrarily designated as the low transfusion group and those receiving more than 10 units as the high transfusion group (Table 2).

Contamination present at the time of admission or surgery was graded as follows: massive soft-tissue contamination or large bowel spillage (2+), minor soft-tissue, intrathoracic or abdominal contamination (1+), or no contamination (0).

Venous blood samples were obtained for immunological studies from each patient within 24 hours of admission and at regular intervals during the hospital stay. Further samples were obtained from all patients during follow-up.

Clinical Infection

Infectious events in these patients have been classified into major/minor groups by usual clinical criteria. Major events include every example of bacteremia, all of which were associated with definable clinical problems, major wound infection, intra-abdominal or other serous cavity abscesses, and any situation in which reoperation for suspected infection was undertaken. Minor events include urinary tract infections and minor wound sepsis.

Some positive cultures of pathogenic bacteria from orifices of sites that, in our judgment, were unassociated with any sign of clinical sepsis were reported but not classified as an infectious event. In the following analysis, those patients who developed major infections (septic) are compared with the group of patients with minor infections and no infection (nonseptic).

Antibiotic Utilization

Antibiotics were generally infused prior to the initial operation and often in the first bottle of intravenous fluids provided upon reaching the hospital. The most common agent used was cefazolin or cefoxitin in 1–2 g doses. The general plan of use in the badly hurt patient included a 5- to 7-day empiric program. Ordinarily, antibiotics would be stopped and the patient recultured if any infectious episode appeared to develop. The plan of empiric, single drug therapy was discarded if and when clinical sepsis appeared earlier than the fifth to seventh day, the drug selection in that case being based on culture data and specific antibiotic sensitivities, where possible. Multiple antibiotic therapy was undertaken only in the presence of specific positive cultures of multiple organisms in gravely ill patients. Reoperation was routinely undertaken when a mechanical problem was identified or suspected, especially in a deteriorating patient.

Respiratory Assistance

Ventilatory support was frequently utilized when major thoracic trauma was a clinical factor. Arterial blood gases were used to monitor the need for and effectiveness of such support. Positive end expiratory pressure, in general, was utilized in preference to increasing the inspired oxygen concentration above 50%. Combinations of continuous positive airway pressure and intermittent mandatory ventilation were used to wean most patients. When airway intubation was required for 2 or more weeks, a tracheostomy was often performed. Only two patients in the group manifested pneumonia as their major septic event.

Nutritional Support

The nutritional status of the patients on admission to the unit uniformly reflected pre-existing normal nutrition. Enteral nutrition was started as soon after admission as the clinical situation allowed; in unconscious patients or those with severe facial injury, it was administered *via* a fine bore nasogastric feeding tube. Twenty per cent of patients received some element of parenteral nutrition according to standard practices during their hospital course. There was no evidence of overt or occult starvation evolving in any of the patients under study.

Injury Severity Score

The Injury Severity Score (ISS) was used as an index of injury severity. To allow more meaningful comparisons between equivalent injuries in patients of different ages, the concept of the 50% lethal dose of injury has been used.[2] The ISS for each patient has been expressed as the percentage LD_{50} for their age.

Flow Cytometry Analysis

Mononuclear cell subset analysis: The per cent and absolute numbers of various lymphocyte subsets were measured in the peripheral blood of study subjects using flu-

TABLE 1. *Patient Data Base*

Age	Race Sex	Mode of Injury	ISS	%LD_{50}	Blood TX 48 hrs	SICU (days)	Hospital (days)	Follow-up (days)	Degree of Contamination (0, 1+, 2+)	Infection	Principle Infection Site	Principle Organisms
29	BM	GSW	34	85	22	5	19	19	1+	None	—	—
60	WM	MVA	41	141	8	9	39	190	1+	Minor	Urine	Gram-negative
52	WM	Impalement	29	100	9	9	37	151	2+	Major	IA abscess	*Streptococcus faecalis* Gram-negative
24	WM	GSW	34	85	24	10	32	129	1+	Major	Blood, urine, sputum	*Pseudomonas aeruginosa* *Staphylococcus aureus*
21	WM	MVA	50	125	6	14	38	180	0	Major	Chronic respiratory tract	*S. aureus* Gram-negative
42	WM	Crush/SPLX	33	83	31	2	16	142	0	Minor	Respiratory tract, urine	Gram-negative
32	WM	Fall	38	95	22	11	40	40	2+	Major	Chronic perineal	Gram-negative
27	BM	SGW	35	88	56	33	93	176	2+	Major	Massive (including eye); chronic soft tissue	*P. aeruginosa* *S. faecalis* Gram-negative
25	BF	SGW	29	73	42	29	53	120	2+	Major	Pneumonia IA abscess	*P. aeruginosa* *P. aeruginosa, S. faecalis*
26	WF	GSW	25	63	15	7	16	159	0	Minor	Urine	*S. faecalis*
26	WM	MVA	41	103	4	14	55	55	0	Major	Chronic respiratory tract, urine	*S. faecalis* Gram-negative
23	BM	MVA	50	125	15	7	55	120	0	None	—	—
17	WM	MVA	29	73	2	4	9	51	0	None	—	—
24	WF	Burn/PG+	50	125	2	16	16	16	2+	Major	Burns, pneumonia	*S. aureus*
24	WF	MVA	34	85	3	5	34	78	1+	Major	IA abscess	*S. aureus* Gram-negative
23	WM	Knife MVA	41	103	13	15	22	74	0	None	—	—
22	BM	GSW	50	125	13	3	11	11	1+	None	—	—
22	BM	Knife	34	85	14	4	9	9	1+	None	—	—
47	BM	GSW/SPLX	25	86	4	3	38	38	1+	None	—	—
25	WM	Crush	20	50	0	6	18	18	0	Major	Blood respiratory tract	*S. aureus*

SPLX = splenectomy, GSW = gunshot wound, SGW = shotgun wound, PG+ = pregnancy, MVA = motor vehicle accident; IA = intra-abdominal.

orochrome-labeled monoclonal antibodies and flow cytometric analysis. Antibodies used and the subsets they identify are listed in Table 3. Monoclonal antibodies were purchased from Becton–Dickinson, Sunnyvale, CA, Orthodiagnostics, Raritan, NJ, and Coulter Immunology, Hialeah, FL.

Forty microliter aliquots of buffy coat cells prepared from acid–citrate–dextrose anticoagulated blood were stained for 20 minutes with the manufacturers' specified dilution of antibody in the presence of 0.1% NaH_3. Red cells were removed by hypotonic lysis, and the samples were preserved in 1% paraformaldehyde. Whole blood was stained in this manner to preclude artefactual distortion of lymphocyte subset distribution often observed in gradient-purified cell preparations.[3]

Samples were analyzed on a cytofluorograph IIS flow cytometer (Ortho Diagnostics, Westwood, MA) configured for simultaneous 2-color (red and green) fluorescent analysis. The forward and right-angle light-scattering properties of cells enabled three-part differentiation of lymphocytes, monocytes, and neutrophils. Fluorescence could thus be measured separately on each cell type. Additionally, Class II major histocompatibility antigen (HLA-DR) was measured on monocytes, which stained positively for MO_2. At least 1000 lymphocytes and 100 monocytes were analyzed in each sample. Complete white blood cell counts (Coulter Counter, Coulter Electronics Inc., Edison, NJ) and Wright-stained microscope differential counts were made to calculate the absolute number of each subset per cubic millimeter of blood.

Please note that all data are being presented as the group or subgroup mean against a normal range of plus or minus *two* standard deviations. This broader interpretation of normal provides a 95% confidence limit for the observations and requires that the investigators show *overtly* dissimilar data before these ranges (which are generally represented by shaded areas across the multiple figures to follow) are exceeded. Such a decision provides protection against the possibility of a type 1 error but creates an increased opportunity to make a type 2 error of obscuring a potentially meaningful interaction or relationship.

Oxidative Burst

The capacity of neutrophils to generate oxidative burst metabolites either endogenously or in response to stimulation with phorbol myristic acetate was measured. Leukocytes isolated from acid–citrate–dextrose anticoagulated blood by hypotonic lysis were labeled internally with dichlorofluroescein diacetate, which in the presence of oxidative burst metabolites emits a green fluorescence that can be detected by flow cytometry.[4] The resting unstimulated generation of fluorescence was measured over a 15-minute period and the phorbol-stimulated generation of fluorescence over a 10-minute period.

TABLE 2. *Patient Data Comparing High and Low Transfusion Groups**

	High Transfusion	Low Transfusion
Number of patients	11	9
Units of blood (48 hours)	24.3 ± 13.8 (13–56)	4.2 ± 3.0 (0–9)
Age (years)	26.8 ± 5.9 (22–42)	32.9 ± 15.7 (17–60)
ISS	36.6 ± 7.8 (25–50)	35.4 ± 10.7 (20–50)
$\%LD_{50}$ ISS	91.8 ± 19.4 (63–125)	98.7 ± 28.7 (50–141)
Hospital days	33 ± 25 (9–93)	32 ± 14 (9–55)
SICU days	11 ± 10 (2–33)	9 ± 5 (3–16)
Major infections	8	6
Minor infections	6	3
Deaths	0	1

* Mean ± SD (range).

Serum Opsonin Capacity

Individual sera samples were tested for their ability to opsonize *Staphylococcus aureus* for phagocytosis by neutrophils obtained from a normal volunteer. Formalin-killed bacteria were stained with a fluorescent dye (Texas red). The stained bacteria were incubated for 30 minutes at 37 C with dilutions of test serum or control pooled human serum. A paired aliquot of serum was heat inactivated at 56 C for 45 minutes to determine whether the opsonic capability of the serum was complement dependent.

The bacteria were washed and incubated for 30 minutes at 37 C with the leukocyte preparation at a bacteria:cell ratio of 10,000:1. Each sample was then washed and fixed in paraformaldehyde. Phagocytosed bacteria were detected by flow-cytometry. Data were expressed as the mean fluorescent intensity emitted from neutrophils that ingested one or more bacteria, normalized to the pooled human serum control. Two thousand neutrophils were analyzed in each sample.

TABLE 3. *Phenotypic Analysis of Mononuclear Cells*

Monoclonal Antibody	*Cell Type Detected*
Single Marker Analysis	
B1	Total B lymphocytes
OK T11	Total T lymphocytes
OK T4 or Leu 3	Helper/inducer T lymphocytes
OK T8 or Leu 2	Suppressor/cytotoxic T lymphocytes
HLA-DR	Class II HLA antigen-bearing cells
MO_2	Monocytes
Leu 7	Natural killer cells
Dual Marker Analysis	
OK T4 + HLA-Dr	Activated helper/inducer
OK T8 + HLA-DR	Activated suppressor/cytotoxic
Leu 2 + Leu 7	Functional suppressor cells
MO_2 + HLA-DR	Antigen presenting monocyte

Materials and Methods

Enzyme Linked Immunosorbent Assay (ELISA)

IgG, IgM, and IgA antibodies to one strain of *Escherichia coli* (O.:K • :NM) and three strains of *Pseudomonas aeruginosa* (O serotypes: M, E, EG) were detected using an enzyme-linked immunosorbent assay (ELISA). The ELISA procedure used was modified from a method developed and validated in our laboratory.[5] The assay was performed in flat-bottomed 96 well polystyrene microtest plates (Flow Laboratories, Inc., Hamden, CT). Formalin-killed bacterial suspensions of the test organisms in carbonate buffer (0.05 M, pH 9.6) were added to the wells in 200 μl aliquots and incubated for 3 hours at 37 C. After the plate was washed with phosphate buffer saline with 0.02% sodium azide added (PBSA), PBSA with 0.5% bovine serum albumin (BSA) was added to each well and incubated for 90 minutes at 27 C. After a second wash with phosphate buffered saline with 0.05% Tween 20® and 0.02% sodium azide (PTA), 200 μl of test serum diluted 1:200 in PTA–BSA was added to each well and incubated for 2 hours at 27 C. After a third wash with PTA, alkaline phosphatase-labeled goat antihuman IgG, IgM, and IgA (Sigma Chemical Company, St. Louis, MO) were added in a 1:1000 dilution in PTA–BSA and incubated for 15–18 hours at 27 C. After a final wash with PTA, 200 μl of nitrophenol substrate (Sigma) 1 mg/ml in carbonate buffer with 0.001 M MgCl was added to each well. The plates were incubated for 60 minutes at 27 C and the reaction then stopped by the addition of 50 μl in 1 N NaOH to each well. The optical density (OD) of each well was measured photometrically at 410 nm with a rapid microplate reader (Mini-reader, Dynatech Laboratories, Inc., Santa Monica, CA). Controls for each plate included wells with antigen but no serum and wells with reference serum and antigen. The control serum was obtained from a healthy laboratory worker documented to have immunoglobulin levels within the normal range as tested by radial immunodiffusion (Kalestead Laboratories, Austin, TX). Patient's serum was tested in quadruplicate for each of the three antibody classes against each of the four organisms and the mean OD recorded.

To minimize intrinsic variation within the ELISA, the following standardization procedure was used. The optical density recorded for the reference serum in the *first* assay performed was designated as a standard and has been used to correct data from all subsequent assays. In cases where the optical density reading was off scale, the assay was repeated using patient's serum and control serum diluted to 1:400 in PTA–BSA.

Cytomegalovirus Antibody Assay

Patient's serum was assayed for antibodies to cytomegalovirus (CMV) on admission, at 2–5 weeks, and at 7–13 weeks. Immunoglobulin IgG antibody to cytomegalovirus (CMV–IgG) was detected and quantified using a solid phase fluorescent immunoassay (FIAX test kit and fluorometer, MA Bioproducts, San Jose, CA). Immunoglobulin IgM antibody to cytomegalovirus (CMV–IgM) was assayed using indirect fluorescent microscopy (CMV–IgM Test Kit, Gull Laboratories, Salt Lake City, UT). The serum used in the CMV–IgM assay was prepared by passage over an ion-exchange resin (Quic-Sep Isolation System, Isolab, Inc., Akron, OH) to remove the IgG fraction.

The appearance of CMV–IgM or CMV–IgG in a previously seronegative patient was taken as evidence of primary CMV infection. Those patients who were seropositive on admission were classified according to whether they exhibited serological changes consistent with active infection during the course of their study. No attempt was made to differentiate between reactivation of a latent infection and reinfection. On the basis of similar assays, we arbitrarily chose a 2.5-fold rise in CMV–IgG FIAX titer as being diagnostic of active infection. The appearance of CMV–IgM during the course of study in a previously seropositive patient was regarded as diagnostic of active infection. In most cases, both criteria were fulfilled. Fifteen patients were tested for CMV on at least two, and in most cases three, occasions.

Skin Testing

Patients were tested with the following skin test antigens shortly after admission to hospital and at weekly intervals during their hospital course or until a positive response was elicited: *Candida albicans* (Center Laboratories, Port Washington, NY), tuberculin purified protein derivative (Aplisol, Park–Davis, Morris Plains, NJ) and trichophyton (Berkeley Biologicals, Berkeley, CA).

Intradermal injections of 0.1 ml of each antigen were administered and read by the same person 24, 48, and 72 hours later. A skin test was considered positive if the diameter of induration was greater than 5 mm for Candida and trichophyton and greater than 10 mm for tuberculin purified protein derivative. Patients were classified as normal if they demonstrated a positive response to any one of the recall antigens. Patients with no positive responses were classified as anergic.

Data Analysis

Initial analysis of the lymphocyte and monocyte data was performed by comparing the means of the patient subgroups at each of the postinjury sample periods and are presented graphically. Some data points were excluded because they did not fall within stipulated time periods. In the final statistical analysis, some of these excluded data points were included if they lay sufficiently close to the stipulated range to permit physiologically valid com-

parisons. Statistical analysis was performed using an IBM 3083 computer (Boca Raton, FL) with SPSSX software (SPSS, Inc., Chicago, IL).

Results

The mean ISS for the study group was 36 (range: 20–50) and the mean $\%LD_{50}$ was 95 (range: 50–141). Figure 1 reflects data calculated to express an LD_{50} for each injury severity score at each of the three truncated ages for which data were available.[2] Consider that the requirement for large amounts of blood transfusion or severe bacterial contamination would tend to shift that curve downward and to the left and that small blood transfusions and bacterial contamination would tend to shift it upward and to the right.

Fourteen major septic events occurred in 10 patients. One patient died as a result of overwhelming burn sepsis complicated by staphylococcal pneumonia. Nine minor septic events were identified. Details of these events are given in Table 1. Only the most severe septic event experienced by any patient is identified. In cases where the patient experienced two separate minor septic events affecting the same system, this has been classed as one event.

Not surprisingly, major infection correlated with significant initial contamination ($p = 0.033$, two tail Fisher's exact test). Major transfusion was not positively correlated with subsequent major infection ($p = 0.37$, two tail Fisher's exact test); further analysis of patients with less than ten and more than 20 transfusions did not disclose a relationship nor did holding the degree of contamination constant. The degree of injury, as measured by the ISS, did not correlate with the development of major infection ($p = 0.89$, one way analysis of variance).

The total leukocyte count in highly transfused patients at the first observation tended to be substantially lower than that in patients who were slightly transfused. This may be explained partially by the observed reduction in polymorphonuclear leukocytes in stored blood.[6] The normal response to injury in this study appears to be an immediate polymorphonuclear leukocytosis, possibly resulting from increased endogenous steroid levels. However, massively transfused patients did not manifest that increase, presumbly for the reason noted above. Patients destined to become infected tended to have lower initial total leukocyte counts, although this was greatly influenced by their transfusion status.

Total T-cells in the population under study are reflected in Figure 2 and represent what is interpreted as a normal and common response to injury and subsequent treatment. All patients had low total lymphocyte counts on admission and exhibited a further decline on day 3; those patients who were highly transfused had relatively lower levels initially. Hematocrit data suggest that only 12–15% of such reduction could be attributed to hemodilution,

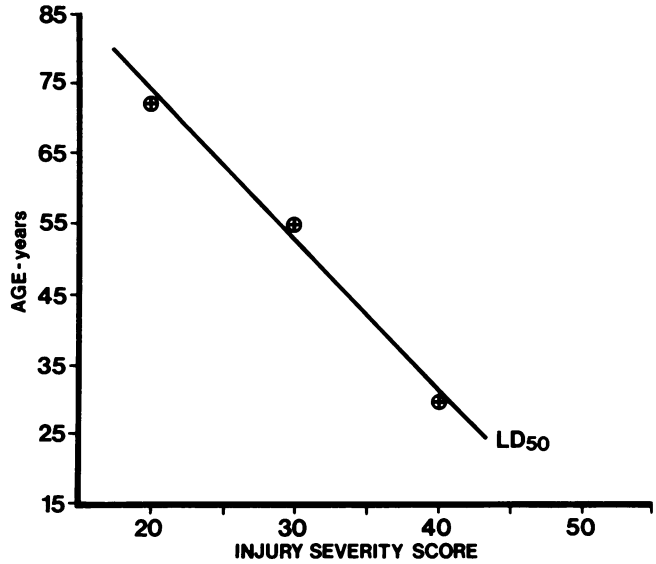

FIG. 1. Injury severity score data calculated to estimate an LD_{50} for each age and ISS point.

assuming that the immunologic cells under study remain within the intravascular compartment and are diluted in parallel with the red blood cells. The major cause of this phenomenon is probably a redistribution of lymphocytes from the circulation to the tissues mediated by endogenous corticosteroids.[7]

Figure 3 presents the total T-cell population normalized for the day of onset of sepsis. The appropriateness of using the time of injury as "time-zero" has been questioned by some, and Figure 2 is an effort to define time-zero arbitrarily as the onset of identifiable clinical sepsis. To some extent, this process is defeated by the need to define arbitrarily the onset of sepsis. The highly variable responses further contribute to misinterpretation. Note that seven of ten such patients approached the septic event with a

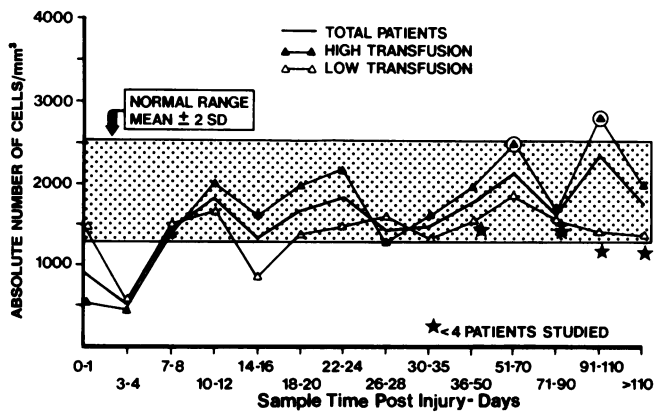

FIG. 2. The normal common response to injury is reflected by the total T-cell observations across the time of the study. Those data points that are circled reflect patients who had undergone splenectomy as part of their prior treatment.

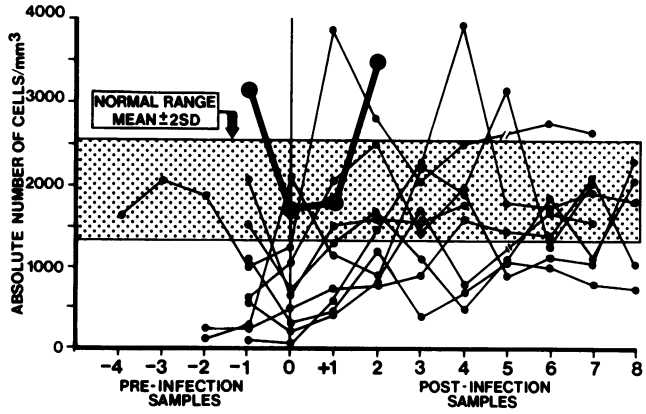

FIG. 3. Total T-cell numbers plotted across time of observation but normalized for empiric designation of day of onset of first infection. The bold line represents the sole septic death.

reduction in total T-cells, whereas three of ten did not. However, nine patients were below normal or in the lower half of the normal range on the documented day of sepsis. The interpretation of this data is obscured by the fact that the majority of infected patients showed their first signs of sepsis on the third day after injury, and it is difficult to tell whether this observation represents a normal response to injury and resuscitation 72 hours previously or a response to a common day of onset of clinical infection. The only useful and direct information we can bring to bear on this is that two of the three patients, who did not become overtly infected until after day three, had very low total T-cell populations on day three; this suggests that low total T-cells are a manifestation of the time after injury rather than specific for the onset of clinical infection.

The T-helper cell population appeared to reflect total T-cell populations. Interestingly, the highest early level of T-helper cells was seen on the seventh to twelfth days after injury, in a patient who had undergone splenectomy with low transfusion as part of the management of multisystem trauma. The highest late value, observed at approximately 50 and 100 days after injury, represents another patient who had undergone splenectomy but who also received 31 units of blood during his initial resuscitation and treatment. It is however, a specific decrease in the T-helper cells that accounts for much of the reduction of total T-cells on the day of admission. An analysis of the T-helper cell population according to the septic or nonseptic status of the patient shows no difference between the groups, as was the case for total T-cell observations. The T-helper cell counts, when normalized for day of onset of sepsis, show that five of ten patients had distinctly low levels prior to the onset of sepsis and six of ten were below normal coincident to the onset of clinically apparent infection.

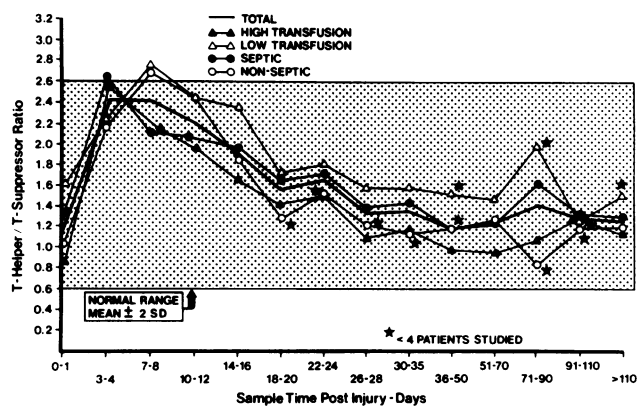

FIG. 4. T-helper/T-suppressor cell ratios as manifested in five groups: total, low, and high transfusion patients, and septic and nonseptic patients.

Observations regarding the T-suppressor cell are similar to those with the total T-cell population. The T-suppressor cell values do not correlate with the presence or absence of clinical sepsis but do appear to correlate better with the relative amounts of blood transfusion. High transfusion tended to be associated with increased levels of T-suppressor cell activity, whereas the slightly transfused patients generally tended to have lower levels. Furthermore, T-suppressor cell activity is more positively related to transfusion than to the presence or absence of CMV infection, although all notable peaks occurred in heavily transfused patients who had serological evidence of active CMV infection.

Figure 4 reflects the ratio of T-helper to T-suppressor lymphocyte activity and is fully compatible with normal recovery from injury. There is a tendency toward a slightly lower level on admission in highly transfused patients. The reduction in T lymphocytes between days one and three is greater within the suppressor population and is reflected in the simultaneous rise in helper/suppressor ratio. Throughout the study period, the ratio remains within or close to the normal range and represents a classic example of homeostasis that is little disturbed by widely varying challenges.

Total B lymphocyte activity waxes and wanes, within or close to a normal range in each of these patients. The only notable exceptions were observed in patients who had undergone splenectomy as part of their management. This elevation was apparent early in one patient who was free of evidence of CMV infection and tended to occur much later in a patient with documented CMV infection.

The antigen presenting capacity of monocytes (MO_2-DR) appears to be very low early on as a function of the injury, operation, anesthesia, or resuscitation involved (Fig. 5). Contrary to all other observed immunologic parameters in this particular study, no decline occurs on the third day after injury, and there is steady improvement toward normal throughout the course studied, although normality is not consistently reached until the 28th day

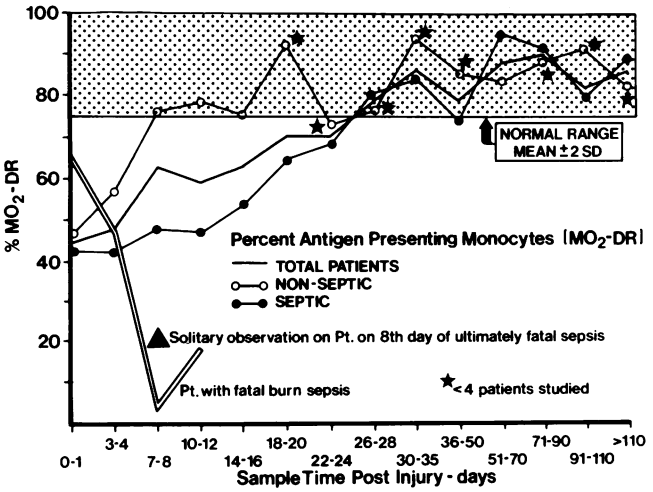

FIG. 5. Septic patients have fewer antigen presenting monocytes during the 4-week period following injury. Note the patient indicated by the solid triangle who died a septic death unassociated with injury but was not otherwise studied repetitively and the course of the patient in whom the sole septic death occurred (open line).

after injury. Interestingly, the nonseptic group and the highly transfused group tended to reach the normal range sooner than the septic and slightly transfused groups. One-way analysis of variance showed a positive correlation between low MO_2-DR and the development or presence of major infection at days 7–8 ($p = 0.013$) and days 10–12 ($p = 0.006$). Similar analysis showed a positive correlation between low MO_2-DR and low transfusion at the same periods ($p = 0.049$ and 0.069, respectively). Two way analysis of variance showed the major effect to be the presence of infection.

More sophisticated analysis is needed to describe interactions between the immunologic parameters under study. Figures 6 and 7 attempt to better define the interaction of the antigen presenting monocytes with total T-cells and T-helper cells. Notice in Figure 6 that, as one moves across the course from the first to the fourth observation, all made in the first 2 weeks after injury, the characterization of septic and nonseptic patients becomes more clear and the dominant role of MO_2 activity over total T-lymphocyte numbers is increasingly clear. Figure 7 describes four discrete quadrants of activity in plotting T-helper cell numbers against MO_2-DR. Quadrant I contains a group of patients who regularly developed infection; no patients reside within quadrant II, which is the boundary for both normal T-helper and normal MO_2 numbers. Quadrant III, which reflects depressed T-helper activity and normal antigen presenting monocyte activity, contains a group of patients who infrequently become infected. Quadrant IV contains a group of patients of whom half become infected. The plot of MO_2-DR against T-helper cells provides a slightly better discrimination at days 2–5 than is possible with the corresponding plot of

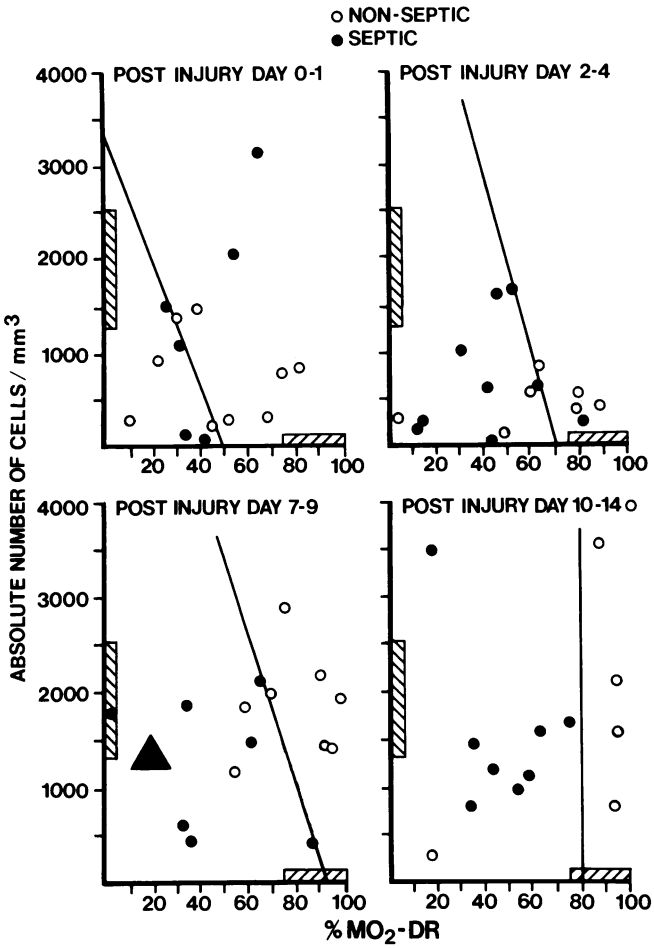

FIG. 6. Plots of antigen presenting monocyte activity (MO_2-DR) *vs.* total T-cells show the increasing significance of the former factor across the first 2 weeks after injury.

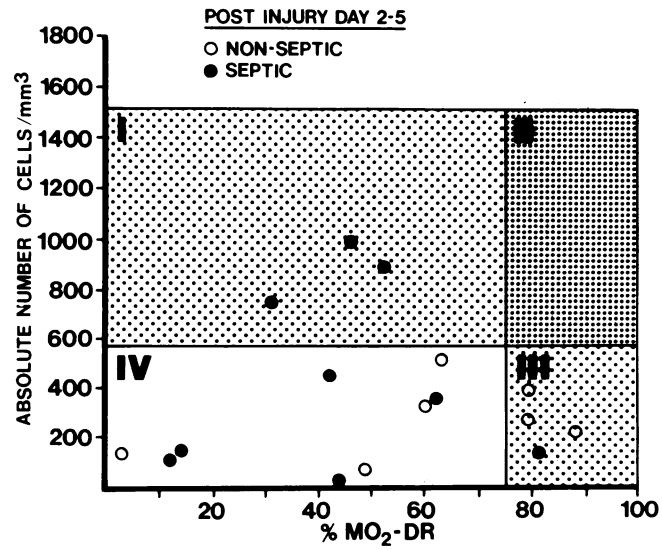

FIG. 7. T-helper cells plotted against MO_2-DR activity on postinjury days 2–5 provide further definition of likely patient status with respect to present on subsequent infection.

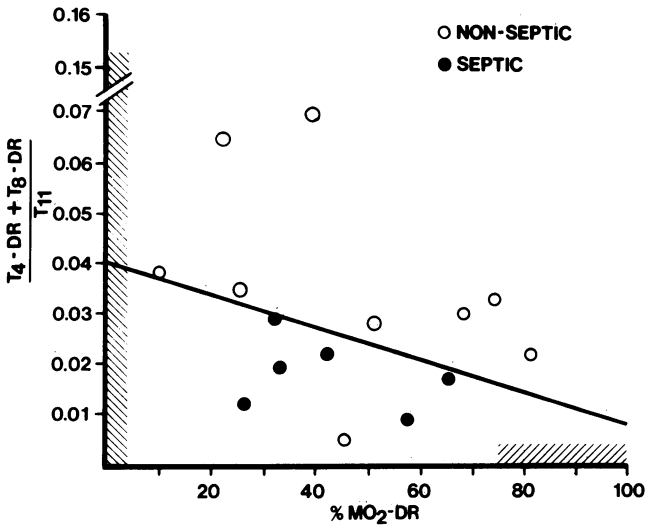

FIG. 8. Analysis on day of admission of the ratio of activated T-cells to total T-cells plotted against MO_2-DR < 24 hours after injury allows identification of patients likely to develop major infection.

MO_2 against total T-cell number. Clearly MO_2-DR is the most useful characterizer of infection among those nonspecific immunologic parameters studied in this data. Predictors are obviously more valuable than characterizers, and Figure 8 reflects an effort to recognize predictive value. This analysis indicates that some variable combination of low ratios of activated T-cells to total T-cell activity and few antigen presenting monocytes are associated with a high rate of subsequent, major infection, even on the very first observations made after the patients were admitted to the trauma service. Clearly, a predictor is much more useful than an overt sign of an event that has already occurred and is demonstrable clinically. If the predictive value of this relationship can be confirmed by others, it would be an uncommonly valuable method for

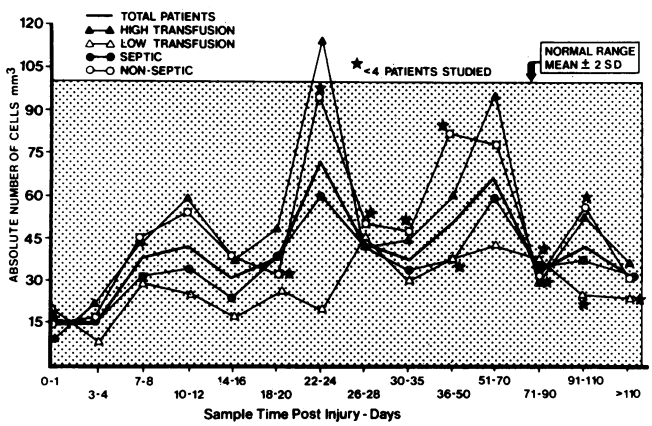

FIG. 9. Absolute number of activated T-helper cells in the five groups characterized as in Figure 4. Highly transfused patients show higher levels of activated T-helper cells, particularly during the second and third weeks after trauma.

early identification of patients who are likely to develop infection as a consequence of their injury.

Natural killer cells usually express T11 surface antigen[8] and are included as a subgroup of the total T-cell lymphocyte population. They exhibit spontaneous cytotoxic activity against both virally infected and tumor cells and lack major histocompatability complex restriction.[9] Our data indicate that the curve across time for those patients who receive few transfusions is low normal. A sequence of late elevations is seen, but these also occur within the normal range and tend to be seen in the patients who had evidence of active CMV infection and/or in highly transfused patients. Such patients are obviously often one and the same. The observed elevations did not appear to relate to clinical septic episodes, a relationship that is more clearly visible when natural killer cell activity is reanalyzed with time-zero set to the onset of clinical infection.

Functional suppressor cells share expression of Leu-7 with the natural killer cell population and again typically reflect a viral infection,[10,11] such as CMV infection commonly seen in these patients. Accordingly, its time course and pattern mirrors that described for natural killer cell activity.

Increased numbers of activated T-helper cells (Fig. 9) tended to reflect lower transfusion state at day one ($p = 0.048$), but high transfusion state at days 10–12 ($p = 0.025$), 14–16 ($p = 0.022$), 18–20 ($p = 0.089$), and 22–24 ($p = 0.067$) by one way analysis of variance. No significant correlation between the number of circulating activated T-helper cells and bacterial or viral infections was found. The increased number of activated T-helper cells seen in the heavily transfused patients 2–3 weeks postinjury may reflect a normal response to major blood transfusion.

Activated suppressor T-cell numbers were similarly studied in these patients. One way analysis of variance showed that activated T-suppressor numbers correlated positively with high transfusion at days 7–8 ($p = 0.066$), days 10–12 ($p = 0.037$), and more strongly at days 22–24 ($p = 0.031$). No relationship existed between activated T-suppressors and CMV infection. Three of the four patients who had chronic bacterial infections showed consistent increases in activated suppressor cell numbers. Each of these cases had their onset at day 3 but literally lasted for weeks; they represent the only example of consistently supernormal activity in the immunologic parameters measured and lead us to suggest that increased activated T-suppressor cell activity is a sign of chronic and/or ongoing bacterial infection as well as major transfusion.

Studies of Opsonization and Phagocytosis

Serial studies of the capacity of patients' serum to support phagocytosis by normal cells were undertaken and

analyzed in detail in those patients who developed bacterial sepsis. This data result is presented in Figure 10. The vast majority of this activity is complement dependent. The serum so used is diluted to 5%, and the parameter shown in this case is the mean channel number, which provides a quantitive measure of the number of labeled killed bacteria ingested by each normal neutrophil; this correlates regularly with the other index measured, the percentage of cells showing phagocytic activity; biologically, these observations are usually one and the same. Three patients in Figure 10 manifested greater than normal capacity of their diluted serum to support opsonic activity than was the case in normal diluted serum. This complement dependent activity unequivocally characterizes the survivors of sepsis. In Figure 10 notice two patients not within the intended scope of the study, for each of whom four data points are available, and the study group's only death, a fatal burn wound sepsis in a pregnant woman. Their sera were associated with reduced capacity to support opsonization. The patient who was 35 weeks pregnant and sustained a greater than 60% body surface area burn in a closed space produced exceptional data in Figure 10, *i.e.*, the capacity of her serum to support opsonization was very low early on and literally improved through the time toward her last observation, which was made 5 days before her final septic episode. Her early illness was characterized by a chronic gram-negative burn wound infection, and her terminal event was characterized by an accentuation of a chronic staphylococcal respiratory infection. The ability of progressive dilution of the serum to display more vividly this unusual characteristic of patients who deal with sepsis successfully and survive is shown in Figure 11.

Heating serum at 56 C for 45 minutes eliminates complement dependent phagocytosis and also serves as an internal control for the process. Most samples in most patients have virtual abolition of opsonic capacity under these circumstances. Notably, one patient's serum appeared to support greater phagocytic activity more often after heating than was seen either in controls or other patients. Three such observations were associated with the onset of clear-cut infectious events in a prolonged course after pancreatoduodenectomy for a shotgun wound of the abdomen. At each such observation, the ELISA determination of Pseudomonas- and *E. coli*-specific IgM and/or IgG were sharply increased. One could hypothesize that such specific immunoglobulins account for residual opsonic activity after heating. However, other patients with similar or higher ELISA values of those same immunoglobulins did not display serum so apparently resistant to heating. These observations are under continuing dissection and may be most germane.

The resting and stimulated capacities of isolated monocytes and polymorphonuclear leukocytes to generate

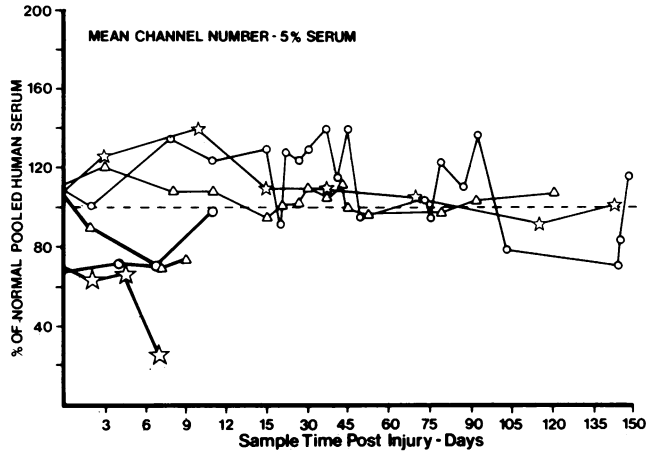

FIG. 10. The capacity of injured patients' serum to support phagocytosis characterizes patients who survive sepsis as well as those who do not. The light lines define three patients who survived major sepsis; the dark lines reflect the one patient in the study who died of infection and two other (uninjured) patients who died septic deaths and for whom only fragmentary data are available.

oxidative burst metabolites were studied in a serial fashion. While there were some impressive peaks that corresponded with defined septic episodes, the absence of such activity in the presence of other septic episodes in other patients was similarly impressive. We conclude that the importance of these observations cannot be stated and that such activity is, at best, erratic.

Enzyme Linked Serum Immunoassay for Specific Gram-Negative Pathogens

This process, utilizing an ELISA developed and validated for both *E. coli* and three strains of Pseudomonas, was incompletely helpful, and further studies are ongoing. The hypothesis that superior immunologic experience prior to injury might favorably influence a badly hurt patient's ability to deal with sepsis cannot be confirmed or refuted at this point in time. However, the ability to increase sharply Pseudomonas- or *E. coli*-specific IgM (or IgG for *E. coli*) was associated with a favorable outcome

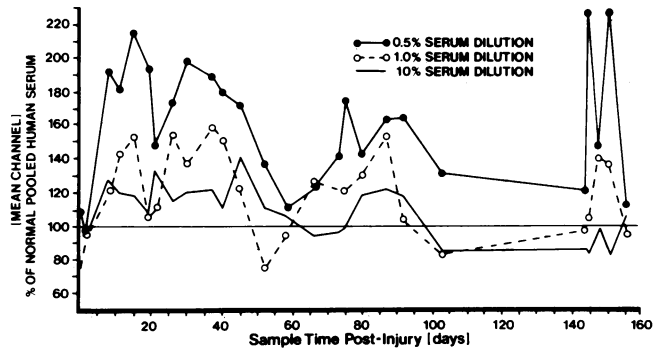

FIG. 11. Progressive dilution of serum more vividly displays the superior capacity for support of opsonization seen in survivors of infection.

TABLE 4. *Number of Patients Demonstrating CMV Infection*

	No Active Infection	Active Infection*	Total
High transfusion	2	7	9
Low transfusion	3	3	6
Total	5	10	15

* Represented by 1 primary infection and 9 patients with reinfection and/or reactivation of latent infection.

with respect to control of major infection in all five patients in which it was noted.

One example may be helpful. A multiply injured patient, but one in whom overt bacterial contamination did not occur, maintained a stormy course in the intensive care unit with no evidence of major identifiable clinical sepsis. He manifested a profound and sustained increase in *E. coli*-specific IgG over this period of time and may be an example of a patient who has dealt successfully with a bacterial challenge without clinical manifestations of such an infection.

A number of patients manifested classic anamnestic responses in which IgG elevations occurred relatively early in their courses, without documented prior significant elevations of IgM, particularly for organisms as common as *E. coli.*

Skin Testing with Recall Antigens

Skin testing data are available for all 20 patients. Two patients manifested normal reactivity on admission to the trauma unit, one of whom developed but survived major infection. Eighteen of the patients were anergic on admission to the hospital, including the sole septic death, a pregnant woman with a large burn. Of those 18 patients who were anergic on initial testing, 12 were available for retesting at 3 weeks or later, three remained anergic, and nine had become appropriately positive. Of those patients who remained anergic, one developed but dealt with a staphylococcal infection well and two remained free of sepsis during the course of their illness. Comparison with the ELISA data previously presented shows that, despite suppositions to the contrary, several patients mounted primary IgM and appropriate IgG responses in the face of continuing skin test anergy.

CMV Results

Serial data points are available on 15 of the 20 patients with respect to CMV infection. Ten patients developed an active CMV infection during the course of their illness, of whom four developed major bacterial infection. Five patients were free of evidence of active CMV infection, of whom three developed major bacterial infection. Table 4 reflects the association of active CMV infection with the degree of blood transfusion. One patient had CMV-IgG and CMV-IgM antibodies in his serum on day one following a 24-unit transfusion. By day 28, CMV-IgM had disappeared, and, by day 99, CMV-IgG had disappeared. This may represent passive transfer of antibody from the donor blood.

Active CMV infection during the first 28 days after injury was significantly associated with the absence of major bacterial infection ($p = 0.041$, two tail Fisher's exact test). Routine testing for CMV seems advisable in any immunologic assay of host defenses in the surgical patient, since it clearly accounts for distortion of the results in many patients. An alternative assessment could be done in patients documented to be seronegative for CMV and transfused with CMV free blood, a situation presently amenable only to elective operations and not to the trauma victim.

Discussion

Sepsis remains the commonest cause of late death following trauma.[12,13] Howard and Simmons stressed the importance of examining the immune system as a whole in the trauma patient and suggested that only by understanding the mechanisms and interactions involved could rational therapy to correct such abnormalities be developed.[14] This study is an attempt to examine multiple aspects of the host defense response to trauma and to define both normal and abnormal responses.

The ISS was used as an index in this study, because it is widely accepted and understood. Both the ISS[1] and the concept of the LD_{50} for given age ranges[2] were derived from data from victims of motor vehicle accidents and have not been validated for other types of trauma. In cases of penetrating trauma, particularly gunshot wounds, its correlation with mortality is poor.[15] A further deficiency of the ISS is that, being an anatomic index, it fails to adequately consider other factors that influence a patient's outcome, such as degree of shock, bacterial contamination, and blood transfusion requirements. A reference to the patients' data base in Table 1 shows only one death in a group of patients with consistently high estimated LD_{50}. We chauvinistically believe this represents good and consistent quality care. On the other hand, it may merely demonstrate the failure of the ISS to reflect adequately the true risk to such patients. We have serious reservations as to the validity of this ISS and to all others as well and feel that an ideal index that combines both anatomical and physiological parameters has yet to be devised.

Interpretation of the changes in lymphocyte subsets following trauma is made difficult because of the effects of both blood transfusion and the frequent development of CMV infections in this group of patients.

The immunosuppressive effects of blood transfusion

were first noted and subsequently used in renal transplantation.[16] More recently, retrospective studies have shown perioperative blood transfusion to be associated with increased recurrence and decreased survival following tumor surgery.[17-19] Blood transfusions have also been implicated in an increased incidence of bacterial infections following elective colonic operations and penetrating abdominal trauma.[20,21] In this study, we did not demonstrate an increased incidence in major infection as a consequence of large blood transfusion. Major transfusion was associated with an increase in the absolute number of circulating T-suppressor cells and increased HLA-DR expression on both T-helper cells and T-suppressor cells. The maximum increase in DR expression occurred between 1 and 3 weeks following transfusion. The appearance in the circulation of atypical lymphocytes during the 3 weeks following blood transfusion may represent a subclinical graft *versus* host reaction to allogenic lymphocytes in the transfused blood.[22] Experimentally, allogenic stimulation increases HLA-DR expression on lymphocytes.[23,24] Clinically, increased HLA-DR expression has been demonstrated in patients receiving repeated blood transfusions,[25] undergoing renal graft rejection,[26] and during acute graft *versus* host disease.[27] This evidence suggests that blood transfusions provide a potent allogenic stimulus that prompts activation of both T-helper and T-suppressor subsets and may represent a subclinical graft *versus* host response. Clearly, this response is occurring in a relatively immunocompetent subject, and in all cases the host triumphs.

We have failed to demonstrate any decrease in the helper/suppressor ratio that has been reported to be associated with repeated blood transfusion, presumably because the stimulation provided by massive blood transfusion is acute and not chronic as it is in those patients receiving repeated blood transfusions.[28]

The mean volume of blood received by patients in this study was 15 units, considerably more than that received by most patients in the literature relating to the immunosuppressive effects of blood transfusion.[29] Clearly, the immunological challenge of a large bolus of blood may elicit a different response than smaller volumes or repeated transfusions over a long period; this may preclude direct comparison of our results with those of others.

The development of active CMV infection following blood transfusion is well documented. In patients exhibiting clinical manifestations of CMV infection, characteristic changes in lymphocyte subsets include a reversal of the normal helper/suppressor ratio resulting predominantly from an increase in the absolute number of T-suppressor cells[30,31] and increases in both natural killer and functional suppressor subpopulations.[10,11] The lymphocyte response to both mitogenic and antigenic stimuli is reduced,[31] but natural killer cell function is unaffected.[30] Our study has confirmed the association between increased natural killer and functional suppressor cell numbers and CMV infection but has failed to demonstrate any significant changes in the T-helper/suppressor ratio or the numbers of T-helpers or T-suppressors. HLA-DR expression on lymphocytes has been shown to be increased in CMV infections.[26] In this study, increased lymphocyte HLA-DR expression was seen in patients with CMV, but the cause of this appeared to be blood transfusion. The absence of some of the lymphocyte changes previously reported may be explained by the subclinical nature of the CMV infections exhibited by this patient group. Reinfections or reactivation of latent infections generally run a less severe clinical course, and this is probably reflected in lesser immunological disturbance. The high rates of active infection found in this study as compared to others are fully consistent with the relatively large volumes of blood transfused, the risk of developing CMV infection being proportional to the volume of blood received. Despite the high incidence of CMV infection in this study, there was no associated increase in major bacterial infection.

There have been relatively few studies of the effects of severe bacterial sepsis on T-cell subsets. In a study of a group of heterogeneous septic patients, multiple T-cell abnormalities were present, the degree of abnormality being proportional to the degree of sepsis. Total T-cells and T-suppressor cell subsets were frequently reduced, T-helper cells were unaffected except in patients over 60 years of age, and the B-cell subset was unchanged in all groups.[32] In this study, B-cells did not respond to severe sepsis, and there were no characteristic changes in the T4 or T8 populations. Severely septic patients with multiple organ system failure showed decreases in the absolute number of total T-cells and T-helper cells in all patients; nonsurvivors were characterized by failure of the total T-cell, total B-cell, and T-suppressor cell populations to rise with therapy.[33] None of these changes were observed in our study, even in our most septic patients. The most characteristic alterations that we noted were rises in the activated T-suppressor cell population during chronic sepsis and the lower lymphocyte number on admission in patients who ultimately developed major sepsis.

Comparison of this study with others examining the effects of operation on T-cell subsets affords some insight into which changes are an intrinsic response to trauma and relatively free of confounding factors of transfusion and CMV and bacterial infection. All studies demonstrated a decrease in the total T-cell population between the first and the third postoperation days and rising to within the normal range again by day 7.[7,34,35] In one study, this decline correlated well with the peak of circulating serum cortisol,[7] and this probably represented a redistribution of lymphocytes to the tissues and bone marrow.

As in this study, the decline in total T-cell numbers was mirrored by changes in the T-helper and T-suppressor populations. The B-cell populations were relatively unchanged by surgical trauma. The T-helper/suppressor ratio response seen in this study, *i.e.*, an initial slight decline followed by a rapid increase always within the normal range, confirms that found previously following operative trauma[34] and multiple trauma without sepsis.[36] Following both thermal and nonthermal injuries, the total T-cell population has been shown to decrease, the decline being more prolonged in thermal trauma.[37] Nonthermal trauma had little effect on T-helper or T-suppressor numbers nor did they change in response to subsequent sepsis. In the thermally injured patients, those with over 30% total body surface area burns had significantly reduced T-helper cells up to 50 days following injury, this effect being most marked in those patients who developed sepsis.

Initiation of an antigen specific T-cell response requires that the antigen be presented to the lymphocyte in conjunction with HLA–DR. Appropriate presentation of antigen to T-helper cells stimulates production of interleukin 2 (IL2) and gamma interferon (IFN-gamma), which facilitate subsequent T- and B-cell responses.

In this study, patients who developed major infections had lower monocyte HLA-DR expression (MO_2-DR) during the 4 weeks following trauma. Whether this represents the cause of the infection or merely a manifestation of it is not clear, although we have shown that patients likely to develop sepsis can be identified very early in their course by plotting the degree of monocyte HLA-DR expression against the ratio of activated (HLA-DR expressing) lymphocytes to total lymphocytes.

Patients with the acquired immune deficiency syndrome (AIDS) have significantly decreased HLA-DR expression on their monocytes.[38] Whether this represents a primary abnormality or is secondary to a reduction in circulating IFN-gamma, resulting from the selective loss of T-helper cells characteristic of the syndrome, is not yet clear.[39] Experimentally, human monocyte HLA-DR expression and murine expression of the equivalent antigen (Ia) is decreased by steroids[40] and prostaglandins.[41] Lipolysaccharide has been shown to inhibit IFN-gamma stimulated monocyte DR expression.[42] In the injured patient, both endogenous glucocorticoids and prostaglandins may contribute to reduced monocyte HLA-DR expression. Further decreases may be caused by blood transfusion, which has been shown to increase prostaglandin E2 production,[43] and the presence of gram-negative bacterial infections, which might cause an increase in the level of circulating liposaccharide. Although HLA-DR expression by monocytes is of crucial importance in effective antigen presentation to T-cells, other factors including monocyte interleukin-1 production appear to be involved.[44,45]

Effective opsonization of bacteria is an essential prerequisite to phagocytosis and intracellular killing. We have studied three patients, one of whom was in the trauma study group, who demonstrated ineffective opsonization during their illnesses, and, in each case, this was associated with a fatal outcome from sepsis. Alexander and associates[46] have shown that severe sepsis may result in a consumption of opsonic proteins such that depletion and subsequent failure of opsonization follows. In that study, the severity of infection was proportional to the degree of complement depletion, and this correlated well with functional assays of the capacity of serum to support phagocytosis and clinical outcome. Independently, we have confirmed that failure of opsonization is an indicator of fatal sepsis.[47] Keusch and associates have similarly described the failure of diluted serum to support opsonization in depleted and/or debilitated individuals.[48] In burn patients it has been shown that, with the exception of properdin, the concentrations of complement components of both classical alternative pathways remain within or above the normal range even in the presence of nonfatal sepsis.[49] However, functional activity of both classical and alternative pathways was depressed during the postburn period, impairment of the alternative pathway being more prolonged. Despite these defects in the complement system, the serum opsonic capacity remained normal, perhaps being effected by heat stable opsonins that were unaffected by trauma, an observation compatible with our demonstration of elevations in heat-stable opsonic activity, which correlated with peaks of immunoglobulin production as measured by ELISA.

Many studies have demonstrated impaired *in vitro* chemotaxis in both thermal and nonthermal trauma.[50–53] However, most of these studies have subsequently shown the defect to reside wholly or partly in the serum of the patient rather than to be an intrinsic cellular defect. Good correlation between impaired chemotaxis and subsequent sepsis was reported in some studies[50,52,53] but was not confirmed by others.[51] Extensive previous studies in this laboratory have failed to show evidence of diminished chemotactic activity *in vitro,* and, for this reason, such studies were not undertaken. Intrinsic cellular chemotactic defects do occur in a spectrum of severe medical disorders,[54] none of which are likely to be operative in patients such as those reported herein. *In vivo* studies of chemotaxis were not done in these patients but will be assessed selectively in future trials.

Our studies of neutrophil bactericidal capacity were undertaken using flow cytometric techniques and measured the capacity of neutrophils to generate oxidative bursts. Gradient separation of neutrophils was not used, since this had previously been found to irreversibly stimulate neutrophil function in this assay. Our results were uniformly inconclusive and failed to correlate with the development of sepsis or other clinical events. We did examine the mean generation of oxidative burst metabolites amongst *all* neutrophils. Recent evidence has sug-

TABLE 5. *Assignment of Value of Parameters Studied with Respect to Their Contributions to the Development of Infection in Injured Patients*

Healthy Response		Unhealthy Response	No Apparent Association	Unknown Effects
Low transfusion		High transfusion	Active CMV infection Total T-cells	
Rapidly normal	Antigen presenting monocytes (MO_2-DR)	Slowly normal	T-helper cells T-suppressor cells	
High MO_2-DR	T4 *vs.* MO_2-DR	Low MO_2-DR	Helper/suppressor ratio	
High T4	Days 2–5 (Fig. 7)		Total B-cells	
High plots	Activated T/total T *vs.* MO_2-DR Day 1 (Fig. 8)	Low plots	Natural killer cells Functional suppressor cells Activated helper cells	
Increased activated suppressor T-cells—chronic infection				
Increased	Opsonic activity in diluted serum (Fig. 10)	Decreased		Ability to retain opsonic activity despite heat inactivation
Sharp increase in IgM in response to infection		Inability to increase IgM in response to infection		Resting monocyte/PMN activity Specific immunoglobulins for *Escherichia coli, Pseudomonas aeruginosa*

gested that the neutrophil population of septic patients is heterogeneous and contains a subgroup of primed cells that have an increased capacity to generate oxidative burst metabolites.[55] Further studies of the proportions of primed and unprimed cells in septic patients and their respective capacities to generate oxidative burst metabolites may prove fruitful.

Both surgery and trauma have been associated with the anergic state as demonstrated by skin testing.[56–58] The degree of anergy was associated with both age and degree of trauma and was alleged to indicate an increased risk of subsequent sepsis.[57] A later study[59] demonstrated that anergy was associated with increasing age, hypoalbuminemia, and reduction in other nutritional parameters but not with subsequent sepsis. In this study, a very high number of patients were anergic on admission, probably reflecting the severity of their injuries. We are unable to confirm the usefulness of skin testing on admission as a predictor of sepsis; however, we did observe specific IgG and IgM responses to gram-negative organisms in anergic patients, indicating that anergy does *not* reflect a global depression of the immune system.

This complex analysis of many parameters in patients who have been badly injured and at major risk for bacterial infection requires careful study, and we have already alluded to the very real possibilities of types 1 and 2 statistical errors in the interpretation of such a complex sequence of observations. Table 5 reflects our conclusions regarding this inquiry and groups the parameters studied with respect to their ability to characterize a healthy or an unhealthy response to injury and contamination or to have no apparent effect or an as yet to be determined effect. We trust that these observations will stimulate others to concentrate their efforts on those inquires, which might tell us more about the meaningful intrinsic abnormalities and responses of the injured patient.

Addendum

Since this paper was completed, six more patients have been studied. Three of these patients have died. Preliminary analysis of the data available on these patients support the hypotheses presented in this paper, particularly those relating to the role of the antigen presenting monocyte as a marker and predictor of infection.

Acknowledgments

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Discussion

DR. PAUL TARTTER (New York, New York): I want to thank Dr. Polk for asking me to discuss this paper. He provided me with copies of the manuscript in draft form and in final form. I was asked to discuss the paper because of my work and interest in blood transfusions and their link to clinical phenomena that we have previously attributed to immune suppression.

We are all aware and were reminded today that blood transfusion prior to kidney transplantation is associated with immune suppression and prolongation of renal allograft survival. In addition, Dr. Polk alluded to some recent studies in the literature linking perioperative blood transfusion at the time of tumor resection to subsequent recurrence of malignancies.

I think the work that really relates to this presentation is that linking blood transfusion to septic complications. There is one study I am aware of that is a multifactorial study of penetrating abdominal trauma where blood transfusion was an independent factor influencing the risk of subsequent infection. In my own work I have found that transfused colorectal cancer patients have twice the risk of developing infectious complications after surgery compared to untransfused patients.

The only experimental work in this field that I am aware of is from the Shriners Burn Unit in Cincinnati, where rats that were subjected to 30% body burn following allogeneic transfusion had impaired immune function, and they also had 2.5 times the septic risk of animals subjected to syngeneic transfusion.

There are many ways that blood transfusions could affect the immune system. It is probable that several different mechanisms are responsible for the observed effects in the different clinical situations. One has to keep in mind that blood banking and blood transfusions have been responsible for significantly lowering surgical mortality in this century, and millions of lives have been saved because of this therapeutic modality. Thus, we need to continue to transfuse patients to replenish blood elements as clinically indicated. We also should continue to study the immune consequences of blood transfusions and of other therapeutic modalities that we commonly employ and that as yet have not been studied.

This is a very important paper. It is the most comprehensive study of immune function in trauma patients to date, and it is the first to relate changes in immune function following trauma to blood transfusion. Further studies of immune function in trauma patients will not be able to ignore blood transfusion as a factor influencing the outcome.

Since this field is well suited to experimental models, I would like to ask the authors if they could tell us about any experimental work they have been doing in parallel to these studies.

DR. JOHN A. MANNICK (Boston, Massachusetts): I too am grateful to Dr. Polk for having provided me with a copy of his manuscript.

Dr. Polk has provided us with an enormous amount of immunological data in the form of serial observations on groups of patients who were seriously injured. What are we to make of all this? My own conclusion is, not much. The reason is that the subgroups are very small in this series. It is an ongoing series, I assume, and we are yet unable to find statistical validity in many of these observations because of the size of the sample population.

A number of laboratories, including our own, have made observations over the years on T-lymphocyte subset alterations after trauma and after burn injury. I agree with Dr. Polk that it is much more difficult to show consistent alterations in T-cell subset ratios after major trauma than it is after major burn injury, where one can deal with a somewhat more cohesive population.

However, I think his conclusion that there is nothing to be learned from T-cell ratios is perhaps wrong, in that the normal variation in his control population was so large that two standard deviations away from the norm would be outside the limits of any observation we have ever made in any patient no matter how sick. I think a more tightly controlled series of normal observations in his lab might allow very different conclusions.

I think that it also might be a mistake to conclude that activation markers on these lymphocyte subsets truly indicate that they are activated. Activation markers are only antigens on the cell surface; and one has to show, before one talks about activation, whether the cells are actually doing something other than making the markers.

(Slide) As you can see here, in burn patients who have activation markers on their suppressor T-cells, the problem is that the suppressor T-cells do not seem to be doing what they are supposed to do, that is, suppressing B-cells from making immunoglobulin. In fact, major burn patients make much more immunoglobulin in response to a standard stimulus than do normal individuals, suggesting that these supposedly activated suppressor T-cells are actually not doing anything at all. Therefore, I am loath to conclude at the moment that the activation markers have great meaning in the population being described today.

I do think that there are some intriguing observations in this data concerning the monocyte activation markers and their relationship with recovery from sepsis, and the question of opsonization. This has been going round and round for many years. Wes Alexander was once very enthusiastic about it; and if I interpret his latest data correctly, Wes no longer believes that measuring opsonic capacity in the serum, at least in major burns, has much predictive value.

I would certainly urge Dr. Polk to continue these studies and try to find whether, in prospective analyses of patients, any of these tests really yield predictive information about the occurence of sepsis.

DR. BASIL A. PRUITT, JR. (Fort Sam Houston, Texas): I apologize for rising for two consecutive papers, but Dr. Polk very kindly provided me with his manuscript, and I would like to compliment him and his colleagues on their detailed examination of the effect of injury on many facets of the immune system.

Of particular interest are their findings of tranfusion-related changes in lymphocyte populations with an early depression and a subsequent rise in leukocyte number in those patients receiving what are termed large transfusion volumes. The findings of a strong association of decreased monocyte antigen presenting capacity and sepsis, a poor correlation of injury severity scores and outcome, and a poor correlation between skin test anergy and infection all help place various assays of immune function in perspective, and emphasize the difficulty in separating cause from effect in clinical studies of sepsis.

Several questions occur, the answers to which will help us interpret these data. First, was the purity of the lymphocyte populations verified histologically, since nonlymphocytes may contaminate subpopulations to a variable extent following injury? This is particularly true in burn patients.

In Figure 7, relating T helper cells and antigen presenting monocytes to infection, the partitioning suggests that higher numbers of T helper cells increase the occurrence of infection, and I wonder whether the small number of cases justifies that implication. Since viral infections in various animal models increase the risk of bacterial infection, the authors attribution of a protective effect to cytomegalovirus (CMV) infections is troublesome. Inasmuch as the CMV titers were strongly associated with transfusion volume, one must ask whether the effect of CMV infection was simply obscured by the transfusion effect?

Lastly, since antibiotics and vasoactive mediators can affect leukocyte function, how were those variables controlled? In fact, the dilution studies for opsonizing activity would suggest that there were circulating inhibitors present rather than some intrinsic change having occurred.

DR. R. FOSTER (Vermont): I enjoyed this paper and would like to focus on the issue of transfusions. We, as well as Dr. Tartter and others, have noted a strong inverse association between the number of blood