The Delayed Hypersensitivity Response and Host Resistance in Surgical Patients

20 Years Later

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Objective

A 20-year follow-up was conducted on research into the implications of a lack of a delayed-type hypersensitivity (DTH) skin test response among surgical patients.

Summary Background Data

The authors' original report showed that a failed DTH response was associated with increased hospital mortality, but the role of specific and nonspecific host defense elements, comorbid factors, nutritional supplementation, and the mechanism for anergy in this adverse outcome was unknown.

Methods

A data base of 4292 patients was analyzed and reported on individual studies designed to answer some of the above questions.

Results

Prospective studies showed a strong association between the DTH response and mortality: reactive patients, 2.9% (75/2576); anergic patients, 20.9% (239/1142, chi square = 265, p < 0.0000001). Antibody response to protein antigens was reduced in anergic patients. Antibody response to polysaccharide antigens was normal in all patients. The hallmark of anergy is a lack of T cells in the skin, as measured by mRNA signal (CD3) for T cells. The nonspecific component of host defense, as measured by circulating and exudate polymorphonuclear cell function, showed no statistically significant difference between elective reactive and elective anergic patients. Notwithstanding some mild malnutrition in anergic patients, parental nutrition failed to correct the DTH response or many of the cellular immune functions measured.

Conclusions

Over the last 5 years, because of a reduction in overall patient mortality, the contribution of a reduced DTH response to septic related mortality has lost statistical significance in elective surgical patients. A reduced DTH response maintains its strong association to sepsis-related mortality in intensive care/trauma patients, and this is the group on which future research efforts should be concentrated.

Twenty years ago, we reported that the lack of a delayed-type hypersensitivity (DTH) response to ubiquitous antigens was associated with increased mortality in surgical patients.[1] Additional reports confirmed these findings,[2-5] although some researchers have questioned their validity.[6] The correlation between an immune dysfunction detected in elective surgical patients before operation and an increased incidence of postoperative mortality among these patients suggested to us a cause-and effect-relationship between these two events. Accordingly, the aim of our investigations has been to gain an understanding of the nature of immune dysfunction in anergic patients as signaled by the lack of DTH reactivity and to understand how this deficit could affect clinical outcome. Our original publication and subsequent literature reports raised several important issues. Does anergy precede infection and subsequent mortality, or is anergy an epiphenomenon? Are there abnormalities in the various components of the immune system (specific B-cell response, specific T-cell function, nonspecific immunity) in anergic patients and do they affect patient outcome? Considering the association between anergy and malnutrition,[7-10] can nutritional manipulation influence host immunocompetence?

To answer some of these questions, we present in the current report our 20 years of research in the field of immunocompetence of surgical patients.

MATERIALS AND METHODS

Patients

The clinical data base consisted of 4292 patients admitted to the wards or surgical intensive care units (ICUs) of the Royal Victoria Hospital, McGill University, from 1973 to 1994. All human experimentation was approved by the Committee on Human Experimentation of the Department of Surgery, Royal Victoria Hospital, Montreal. Patients on the wards were admitted for elective surgical for major gastrointestinal, gynecologic, or urologic resections for cancer, inflammatory bowel disease, gastrointestinal bleeding (stabilized), complicated hepatobiliary procedures, or major vascular reconstructions. The patients in the surgical ICU were admitted for trauma, gastrointestinal bleeding (acute), intra-abdominal infection, or other postsurgical complications. The diagnosis of presumed infection (whether

the patient presented with such infection or the infection developed subsequent to the skin test) was pursued aggressively in an attempt to confirm or exclude its presence by appropriate cultures of blood or suspected infected material. The cause of hospital mortality was determined for all patients. No attempt was made to standardize treatment in these studies, such as type of surgery or choice of antibiotics.

Skin Tests

All patients were skin tested with five ubiquitous antigens within 24 hours after their admission. Additional data were collected as per individual study protocol. Delayed-type hypersensitivity skin tests were performed as follows. A volume of 0.1 mL of each of the following antigens was injected intradermally on the upper arm or forearm. *Candida* (1:100 dilution, Hollister-Stier Laboratories, Mississauga, ON), mumps skin test antigen (undiluted, Eli Lilly Co., Indianapolis, IN), purified protein derivative (5 TU/0.1 mL, Connaught Laboratories, Willowdale, ON), trichophyton, and varidase (both were obtained at 100 U/mL from Hollister-Stier Laboratories). The diameter of the resulting induration was measured at the two widest points at 24 and 48 hours after injection and averaged, and the larger value was then recorded as the individual antigen response in millimeters. The surrounding erythema was ignored. A positive antigen response was defined as a diameter of induration equal to or greater than 5 mm. Patients were classified as *reactive* if they responded to two or more antigens, *relatively anergic* if they responded to one antigen, or *anergic* if they showed no response to any of the antigens. Alternately, a skin test score was recorded as the sum of the diameters of the individual antigen responses.

Definition of an Infectious Challenge

An *infectious challenge* was judged to be present if a culture-proven bacteremia was diagnosed or an intra-abdominal infection was confirmed by an intervention (surgery or percutaneous drainage) that provided pus which subsequently grew a pathogen on culture.

Comorbid Factor Analysis

To determine comorbid factors along with anergy, between 1990 and 1992, we studied 249 patients admitted for elective surgery, as described above. All patients underwent radiologic and laboratory investigations to exclude the presence of a preexisting infection. In addition, a detailed cardiac, respiratory, renal, and hepatic assessment was made. Delayed-type hypersensitivity skin testing was performed. Laboratory data (hemoglobin, circu-

lating polymorphonuclear neutrophil, lymphocyte, monocytes, platelet counts, creatinine, bilirubin), immunologic data (C3, CH50, serum immunoglobulins), anthropometric data (weight, height, weight loss, creatinine-height index, triceps skin fold, midarm circumference), acute phase proteins (prealbumin, retinol binding protein, transferin, C-reactive protein, α-1-antitrypsin, α-2-macroglobulin), and, as an index of malnutrition, body composition analysis by total body impedance plethysmography[11] were recorded. Pulmonary function tests were also performed.

Nutritional Manipulation Studies

To assess the role of nutritional manipulation on host defense, we evaluated three patient groups using a prospective, randomized, and blinded study design. *Group I* consisted of 15 anergic patients admitted for elective gastrointestinal resections. With this group, we asked whether we could abolish anergy with a nutritional supplement containing an amino acid mixture specifically formulated to enhance immune responsiveness. In addition to diet as tolerated, which was limited to 20 g protein per day, the patients were randomized to receive 2 weeks of a regular amino acid solution (8.5% Freamine III; Kendall-McGaw Laboratories, Irvine, CA) or a modified amino acid solution specifically formulated to enhance immune responsiveness, providing 1.5 to 2.0 g amino acids/kg body weight per day in 5% to 25% dextrose (composition of this amino acid formulation is on file). The amino acids were given peripherally in 5% to 10% glucose for patients with exchangeable sodium (Na_e)/exchangeable potassium (K_e) less than 1.2 (minimally malnourished) or in 17% to 25% glucose through the central vein for patients with Na_e/K_e greater than 1.3. *Group II* consisted of 59 reactive patients admitted for major elective gastrointestinal resections. They were randomized to either amino acid infusion in 10% glucose for 7 days after surgery, with infusion initiated in the recovery room. *Group III* included 21 patients admitted to the surgical ICU because of multiple trauma or infection with a systemic inflammatory response. They were randomized to receive either amino acid solution in 17% to 25% glucose through the central vein for 7 to 21 days. Besides the measurements described for the comorbid factor analysis, lymphocyte blastogenic responses were measured using three concentrations of the mitogens phytohemagglutinin and pokeweed and the antigen tetanus, as described elsewhere.[12] Lymphocyte subsets were measured using flow cytometry. Neutrophil adherence and polymorphonuclear chemotaxis were measured as described previously.[13]

B-Cell Antibody Response

Twenty-six healthy control subjects as well as 11 elective reactive, 9 elective anergic, 8 surgical ICU anergic and 12 surgical ICU reactive patients were observed in tetanus toxoid immunization studies. Eight healthy control subjects as well as five elective reactive and seven elective anergic patients were immunized with Pneumovax, as described previously.[14] All vaccinations were given intramuscularly after classification by skin testing, and the *in vivo* antibody response was measured 14 days later by radioimmunoassay. Antibody responses were expressed as the ratio of preimmunization compared with postimmunization sera. Group results were averaged as geometric means, and the data are shown as mean chi square value divided by SEM of the logarithms in base 10.

Specific T-Cell Response

Analysis of cytokine gene expression in biopsies taken from uninjected skin or from skin injected 4 or 24 hours earlier with specific or irrelevant antigen was performed by polymerase chain reaction (PCR), as described by Dallman at al.[15] Briefly, total RNA was prepared from skin biopsies (3.5-mm in diameter) by homogenization in guanidine thiocyanate and centrifugation through cesium chloride. cDNA from the RNA was prepared by reverse transcriptase. For the PCR, oligonucleotide primers and internal oligonucleotides were synthesized for interleukin (IL)-1 α and β, tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-β, IL-2, IL-3, IL-4, IL-6, IL-8, IL-10, and interferon (IFN)-γ. In preliminary experiments that used RNA prepared from peripheral blood mononuclear cells, optimal conditions for the detection of mRNA by PCR for each cytokine were established. Polymerase chain reaction for each cytokine yielded a single band on electrophoresis of the expected molecular size, which hybridized only with the specific internal oligonucleotide. For skin, cDNA from 0.7 to 2.0 μg total RNA was used for each analysis. To quantitate the results, we followed the procedure of Leung et al.[16] ^{32}P-dCTP was used in the PCR reaction mixture. We subjected the PCR product to electrophoresis in agarose, excising the band visualized under ultraviolet light (Fig. 1) and counting the radioactivity incorporated in a scintillation counter. In each experiment, aliquots were removed from each PCR mixture after 30, 35, 40, and 45 cycles of amplification to verify that plateau conditions had not been reached. Typical examples showing a linear increase in incorporation through 45 cycles of amplification for individual cytokines from a DTH-reactive patient are shown in Figure 2. The results were normalized to the counts for mRNA for β-actin control and analyzed

Figure 1. Electrophoretogram of PCR products for quantitative analysis. Leftmost lane contains DNA molecular-weight markers. PCR was carried out for 30, 35, 40, and 45 cycles with primers for (left quartile) IL-1β, (middle quartile showing no mRNA) IL-8, and (right quartile) β-actin control.

similarly for each skin biopsy. The same PCR procedure was used to look for T cells in uninjected skin, with primers used for the epsilon chain of the CD3 molecule.

Nonspecific Host Defense

A detailed analysis of nonspecific host defense was performed in 20 elective reactive and 16 elective anergic patients through measurement of polymorphonuclear neutrophil (PMN) exudation to blister-type skin windows[17] and correlation of exudation to PMN surface adhesion molecules, chemotactic receptors, lipopolysaccharide receptors, superoxide anion generation, and candidicidal capacity. The same measurements were made in circu-

lating PMNs and compared with exudate PMNs by means of techniques described in detail elsewhere.[18]

Statistical Methods

Arithmetic data are represented as means ± SD unless otherwise stated in the text. Continuous variables between two groups were compared with the unpaired Student's t test and geometric data with the Wilcoxon's rank-sum test. Multiple group data were tested for statistically significant differences by means of an analysis of variance and Bonferroni's correction for multiple comparisons. Frequency tables were set up, and two-way comparisons were tested for significance by means of chi square. Log-linear models were set up with BMDP Dynamic (4F; BMDP Corp., Cupertino, CA), and logistic regression analysis was performed similarly performed.

RESULTS

Demographic Data

Patients in this report were observed over a 21-year period, from July 1, 1973, to June 30, 1994. Their demographics and admission diagnosis are listed in Table 1. Of the 4292 patients observed over this period, 2403 were men and 1889 were women, with an age of 61.2 ± 16.5 years. The overall mortality of this group was 11.1%. There were 3081 elective admissions to the surgical wards. The overall mortality rate of the elective patient group was 5.5%. In addition, 1201 patients were admitted to the surgical ICU as emergency cases. They were treated mainly for blunt and penetrating trauma, gastrointestinal bleeding, or established infection. The overall mortality rate of the surgical ICU group was 22.3%.

Outcome Analysis: All-Cause Mortality

All-cause mortality analysis of the whole data base is shown in Table 2. Reactive patients had a mortality rate of 3.1%; relative anergy patients, 11%; and anergic patients, 25.1% (chi square = 415.6, p <

Figure 2. Injection of mumps 4 hours earlier into the skin biopsy from a DTH-reactive patient shows incorporation of ^{32}PdCTP into DNA after PCR through 45 cycles of amplification by means of IL-1β and β-actin primers.
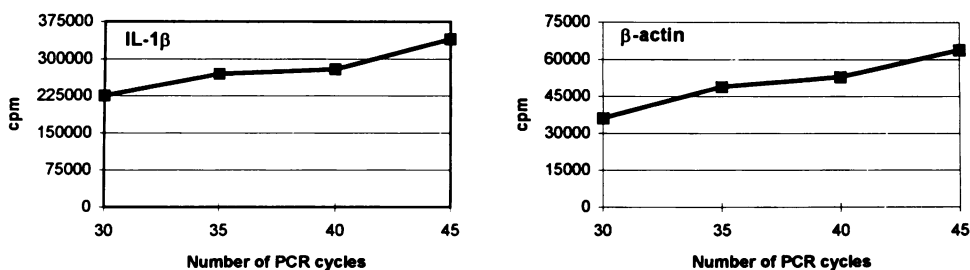
Table 1. DEMOGRAPHIC DATA OF THE 4292 PATIENTS (1973–1994)

Elective patient group (n = 3081)
  1645 men/1436 women
  Age = 62.9 ± 15.5 yr
  Diagnosis

Chronic cholecystitis	174
Chronic pancreatitis	150
Colorectal malignancy	730
Esophageal malignancy	145
Gastric malignancy	278
Gastrointestinal bleeding	220
Genitourinary malignancy	120
Gynecologic malignancy	90
Head and neck malignancy	55
Hepatobiliary malignancy	230
Inflammatory bowel disease	270
Miscellaneous	190
Peptice ulcer disease	295
Peripheral vascular disease	69
Pulmonary malignancy	65

ICU/trauma patient group (n = 1211)
  758 men/453 women
  Age = 58.6 ± 18.6 yr
  Diagnosis

Blunt trauma	220
Diffuse peritonitis	75
Gastrointestinal malignancy	73
Gastrointestinal bleeding	230
Hepatobiliary infection	35
Inflammatory bowel disease	88
Intra-abdominal abscess	258
Miscellaneous	38
Penetrating trauma	120
Peripheral vascular disease	74

0.000001). Log linear modeling showed a strong interaction between an infectious challenge, skin test response, and outcome. The analysis of the 3081 elective patients, for whom preexisting infection was carefully excluded, showed that preoperative anergy was clearly associated with increased mortality, 17.6% compared with 2.1% in the reactive patients (chi square = 210.2, p < 0.00001). The same association between admission skin test response and outcome was confirmed in the 1211 patients admitted to the surgical ICU.

Outcome Analysis: Sequential Skin Testing

A subgroup of 1965 patients underwent weekly sequential skin testing ranging from 3 to 20 weeks. Patients who were always reactive during this time showed a very low mortality rate of 1.4% (21/1496), compared with those who remained anergic throughout this period, with a mortality rate of 55.6% (273/763, chi square = 527.4, p < 0.00000001).

Effect of Infection

The analysis presented in Table 3 shows the strong association between the development of an infection and mortality in the overall data base. The relation between the DTH skin-test response and an infectious challenge was examined in the elective patient subgroup. These patients underwent a thorough search for overt or occult infection before skin testing and were excluded from the study if such an infection was found. Some of these patients subsequently developed an infectious challenge after major surgery. Patients who were anergic before operation and who subsequently experienced an infectious challenge had a mortality rate of 58.2% (32/55), compared with a mortality rate of 12.3% (9/73) for reactive patients.

Table 2. ALL CAUSE MORTALITY ANALYSIS OF THE WHOLE DATABASE (1973–1994) OF 4292 PATIENTS STRATIFIED BY THE ADMISSION SKIN TEST RESPONSE

Skin Test	All Patients* (n = 4292)		Elective Patients† (n = 3081)		SICU/Traum Patients‡ (n = 1211)	
	Alive	Dead	Alive	Dead	Alive	Dead
Reactive	2432 (96.9)	77 (3.1)	2094 (97.7)	46 (2.1)	338 (91.6)	31 (8.4)
Relative anergy	593 (89.0)	73 (11.0)	376 (95.4)	18 (4.6)	217 (79.8)	55 (20.2)
Anergy	837 (74.9)	280 (25.1)	451 (82.4)	96 (17.6)	386 (67.7)	184 (32.3)

Values are no. (%).
* Chi square = 415.6, p < 0.0000001.
† Chi square = 210.2, p < 0.0000001.
‡ Chi square = 74.6, p < 0.000001.

Table 3. THE EFFECT OF AN INFECTIOUS CHALLENGE ON THE CLINICAL OUTCOME OF ALL 4292 PATIENTS (1973–1994) STRATIFIED BY THEIR ADMISSION SKIN TEST RESPONSE

Skin Test	Infection	Alive	Dead
All patients (n = 4292)			
Reactive	No	2293 (98.1)	44 (1.9)
	Yes	139 (80.8)	33 (19.2)
Relative anergy	No	519 (93.2)	38 (6.8)
	Yes	74 (67.9)	35 (32.1)
Anergy	No	692 (83.9)	133 (16.1)
	Yes	142 (49.7)	147 (50.3)
Elective patients (n = 3081)			
Reactive	No	2030 (98.2)	37 (1.8)
	Yes	64 (87.7)	9 (12.3)
Relative anergy	No	361 (96.0)	15 (4.0)
	Yes	15 (83.3)	3 (16.7)
Anergy	No	428 (87.0)	64 (13.0)
	Yes	23 (41.8)	32 (58.2)
SICU/trauma patients (n = 1211)			
Reactive	No	263 (97.4)	7 (2.6)
	Yes	75 (75.8)	24 (24.2)
Relative anergy	No	158 (87.3)	23 (12.7)
	Yes	59 (64.8)	32 (35.2)
Anergy	No	264 (79.3)	69 (20.7)
	Yes	122 (51.5)	115 (48.5)

Values are no. (%).

Comorbid Factor Analysis

One hundred fifty-six men and 93 women (mean age, 63.4 ± 13.3 years) were used in the comorbid factor analysis study. Table 4 shows that anergic patients were slightly older. Anergic patients had measured decreases in forced expiratory volume at 1 second, forced vital capacity, and peak end-expiratory flow. This manifested clinically as a higher proportion of anergic patients with dyspnea on exertion (29.3%) compared with reactive patients (2.5%). The body mass index of anergic patients was lower, and their Na_e/K_e ratio was higher, indicating a mild degree of malnutrition. Anergic patients were anemic, with lower lymphocyte counts, and had lower serum albumin and prealbumin levels but an increased α-2-macroglobulin level. The mortality rate of this comorbid analysis study (1990–1992) was only 1.6%.

Mortality Analysis by Decade

The surprisingly low fatality rate of the comorbid study prompted us to reexamine our mortality data over the past 20 years. Table 5 shows the frequency table analysis of this mortality by skin test and decade. The overall mortality rates were 11.4% up to December 31, 1979;

10.2% up to December 31, 1989; and 7.8% up to December 31, 1994. This overall reduction in mortality rates was reflected in the reactive and anergic patients. The progressive reduction in mortality of anergic patients was primarily due to the lower fatality of elective anergic patients (23%, 25.1%, and 6.3%, respectively) compared with the surgical ICU anergic patients (34.4%, 35.2%, and 28.3%, respectively). Log linear modeling confirmed this interaction. Outcome by decade chi square analysis was 27.4 (p < 0.000001) for elective compared with 3.46 (p = 0.17) for surgical ICU anergic patients.

The logistic regression equation was as follows:

$$P_{|mortality|} = 1 - \left\{ \frac{1}{1 + e^{[(1.3-1.4(albumin)-1.7*ln(DTH) + 0.002(age)]}} \right\}$$

This equation, generated with data between 1974 to 1983, accurately predicted the number of deaths up to 1989 but overestimated the mortality between 1989 and 1994. For example, 18 deaths were predicted in the 249 patients used in the comorbid analysis study. As reported earlier, only 4 deaths occurred (1.6% mortality rate).

Nutritional Manipulation of the Immune Response

The demographic parameters of the three study groups used in the nutritional manipulation experiments are shown in Table 6. Table 7 lists the data for the preoperative anergic patient group. None of the extensive measurements performed were altered by administration of regular parenteral nutritional supplementation for 2 weeks or parenteral nutritional supplementation containing an amino acid solution found to quickly reverse (within 7–10 days) severe malnutrition induced anergy in a rat model. As expected with the preoperative reactive patient study, the DTH skin-test response, PMN adherence, PMN chemotaxis, and acute-phase response, proteins were all altered by elective surgery (controlled surgical trauma). Neither of the parenteral nutritional supplementation regimens was able to influence these changes (data available on file). Similarly, none of the immune-function measurements performed on the surgical ICU patient group were affected by 2 weeks of nutritional manipulation (data available on file).

In Vivo Antibody Responses

Whereas 65% of healthy immunized individuals and 47% of DTH reactive patients made significant amounts of antibody to tetanus toxoid, only 19% of elective anergic patients did so (chi square = 13.7, p < 0.0001). Among the responders, the amount of anti-

Table 4. AGE, RESPIRATORY, NUTRITIONAL, HEMATOLOGIC, HEPATIC, IMMUNOLOGIC, ACUTE PHASE RESPONSE, AND RENAL VARIABLES IN THE 249 ELECTIVE SURGERY PATIENTS (1990–1992) USED FOR THE COMORBID FACTOR STUDY

Variable	Reactive (n = 196)	Relative Anergy (n = 33)	Anergy (n = 20)	p Value
Age (yr)	62.0 ± 13.4	67.4 ± 10.4	70.8 ± 10.8	0.0029
Respiratory				
FEV$_1$ (L/min)	2.3 ± 0.9	2.3 ± 0.9	1.3 ± 0.5	0.0012
FVC (L/min)	3.4 ± 1.0	3.2 ± 1.1	1.9 ± 0.6	0.0000
PEF (L/min)	290 ± 155	303 ± 190	171 ± 88	0.0223
Nutritional				
Weight loss (kg)	3.8 ± 7.2	3.5 ± 4.6	5.3 ± 5.6	NS
Body mass index	22.2 ± 8.1	24.4 ± 3.4	16.9 ± 12.1	0.0050
Creatinine height index	0.57 ± 0.18	0.58 ± 0.11	0.64 ± 0.30	NS
Triceps skin fold (mm)	12.8 ± 6.6	11.9 ± 5.9	12.6 ± 7.8	NS
Mean arm circumference (cm)	29.8 ± 4.2	29.5 ± 3.1	27.9 ± 4.8	NS
Na$_e$/K$_e$	1.1 ± 0.2	1.1 ± 0.2	1.3 ± 0.2	0.0015
Hematologic				
Hemoglobin (g/L)	128 ± 20	125 ± 18	115 ± 17	0.0130
PMN (× 10^9/L)	5.1 ± 4.7	4.7 ± 2.9	5.3 ± 2.8	NS
Lymphocytes (× 10^9/L)	1.70 ± 0.94	1.19 ± 0.39	0.98 ± 0.50	0.0001
Monocytes (× 10^9/L)	0.52 ± 0.35	0.52 ± 0.37	0.46 ± 0.17	NS
Platelets (× 10^9/L)	287 ± 122	269 ± 78	279 ± 83	NS
Hepatic				
AST (U/L)	27.8 ± 29.1	33.8 ± 68.6	35.0 ± 49.6	NS
ALT (U/L)	35.7 ± 72.5	37.2 ± 90.1	34.6 ± 49.0	NS
PTT (sec)	29.1 ± 6.5	29.2 ± 5.5	29.9 ± 2.4	NS
Serum bilirubin (μmol/L)	17.8 ± 15.5	26.5 ± 16.5	43.8 ± 19.0	NS
Acute phase response				
Serum albumin (g/L)	35.4 ± 5.5	33.8 ± 4.0	31.2 ± 6.0	0.0055
α1-Antitrypsin (g/L)	2.6 ± 0.9	2.7 ± 0.8	3.1 ± 0.9	NS
CRP (g/L)	21.9 ± 43.3	24.8 ± 32.5	20.7 ± 28.2	NS
Prealbumin (g/L)	0.23 ± 0.08	0.19 ± 0.06	0.19 ± 0.09	0.0021
Retinol binding protein (g/L)	0.04 ± 0.01	0.03 ± 0.01	0.05 ± 0.05	NS
Transferrin (g/L)	2.5 ± 0.6	2.4 ± 0.6	2.4 ± 0.8	NS
α2-Macroglobulin (g/L)	1.9 ± 0.7	2.2 ± 0.9	2.5 ± 0.9	0.0101
Total protein (g/L)	66.2 ± 7.2	63.3 ± 6.5	62.7 ± 10.0	0.0293
Immunologic				
IgA (g/L)	2.9 ± 1.4	2.8 ± 1.2	3.3 ± 1.4	NS
IgG (g/L)	12.1 ± 3.2	11.3 ± 2.8	12.6 ± 5.8	NS
IgM (g/L)	1.8 ± 1.9	1.5 ± 1.2	1.9 ± 2.7	NS
C3 (g/L)	0.81 ± 0.18	0.90 ± 0.30	0.84 ± 0.26	NS
C4 (g/L)	0.31 ± 0.10	0.35 ± 0.12	0.35 ± 0.12	NS
Renal				
Serum creatinine (μmol/L)	94 ± 23	97 ± 21	104 ± 54	NS

NS = not significant; FEV$_1$ = forced expiratory volume in 1 minute; FVC = forced vital capacity; PEF = PMN = polymorphonuclear neutrophil leukocytes; AST = aspartate aminotransferase; ALT = alanine transferase; PTT = partial thromboplastin time; CRP = C-reactive protein.

body made was least among the anergic patients in the elective and the surgical ICU groups (Table 8). In contrast, healthy control subjects and patients of all skin-test categories made comparable amounts of polysaccharide antibody, either IgG (Table 8), IgM, or IgA (data available on file).

T-Cell Responses

To determine whether anergy could result from a deficit in cytokine synthesis in the skin, using PCR, we took skin biopsies from 53 reactive and 18 anergic individuals for analysis of gene activation for 11 cytokines. Whereas this highly sensitive method does not detect the actual presence of cytokines, the selective absence of mRNA for any one cytokine from anergic, but not from DTH reactive skin, can indicate the absence of its synthesis. Of the 11 cytokines studied, 6 regularly expressed mRNA in skin of DTH-reactive patients. Remarkably, among these patients, the expression of mRNA for the inflammatory cytokines TNF-α and IL-1β and for the

Table 5. ALL CAUSE MORTALITY BY DECADE, FOR ALL THE PATIENTS, STRATIFIED BY THEIR LOCATION IN HOSPITAL AND THEIR ADMISSION SKIN TEST RESPONSE

Skin Test	Period	Alive	Dead
All patients			
Reactive	1973–1979	1064 (96.2)	42 (3.8)
	1980–1989	658 (96.9)	21 (3.1)
	1990–1994	710 (98.1)	14 (1.9)
Relative anergy	1973–1979	194 (84.3)	36 (15.7)
	1980–1989	146 (91.2)	14 (8.8)
	1990–1994	253 (91.7)	23 (8.3)
Anergy	1973–1979	273 (69.6)	119 (30.4)
	1980–1989	219 (71.3)	88 (28.7)
	1990–1994	345 (82.3)	73 (17.5)
Elective patients			
Reactive	1973–1979	877 (97.8)	19 (2.1)
	1980–1989	611 (97.1)	18 (2.9)
	1990–1994	606 (98.5)	9 (1.5)
Relative anergy	1973–1979	106 (93.0)	8 (7.0)
	1980–1989	111 (94.9)	6 (5.1)
	1990–1994	159 (97.5)	4 (2.5)
Anergy	1973–1979	109 (76.8)	33 (23.2)
	1980–1989	149 (74.9)	50 (25.1)
	1990–1994	193 (93.7)	13 (6.3)
SICU/trauma patients			
Reactive	1973–1979	187 (89.0)	23 (11.0)
	1980–1989	47 (94.0)	3 (6.0)
	1990–1994	104 (95.4)	5 (4.6)
Relative anergy	1973–1979	88 (75.9)	28 (24.1)
	1980–1989	35 (81.4)	8 (18.6)
	1990–1994	94 (83.2)	19 (16.8)
Anergy	1973–1979	164 (65.6)	86 (34.4)
	1980–1989	70 (64.8)	38 (35.2)
	1990–1994	152 (71.7)	60 (28.3)

Values are no. (%).

4 hours after injection of antigen revealed T cells in all 16 biopsies tested. In contrast, such analysis based on biopsies from 8 anergic patients was uniformly negative for CD3 molecules, signaling the absence of T cells in the skin of anergic subjects (Fig. 3).

Nonspecific Host Defense

Table 10 shows the detailed analysis of circulating and exudate PMN function in 20 DTH-reactive and 16 anergic elective surgery patients. There was no difference in the number of PMNs delivered to the blister-type skin windows between anergic and reactive patients. No statistically significant differences were detected in CDllb (adhesion molecule), FMLP-r (chemotactic receptor), CD14 (lipopolysaccharide receptor), CD16 (opsonin receptor), superoxide anion generation (at 1 μM FMLP stimulation), or candidicidal activity of circulating or exudate PMNs in anergic or DTH-reactive patients. The process of PMN exudation primed PMNs, as expected. However, no significant difference was detected between the reactive and anergic patients.

DISCUSSION

Failure to produce a DTH reaction is associated with increased mortality in surgical patients. This applies to

Table 6. THE DEMOGRAPHIC PARAMETERS OF THE THREE STUDY GROUPS USED IN THE NUTRITIONAL MANIPULATION STUDIES

Group 1 nonoperated anergic patients (n = 15)	
Men	7
Women	8
Mean age (± SD) (yr)	67.9 ± 8.2
Diagnosis	
Esophageal malignancy	1
Gastric malignancy	4
Hepatobiliary malignancy	4
Colonic malignancy	6
Group 2 preoperative reactive patients (n = 59)	
Men	37
Women	22
Mean age (± SD) (yr)	61.5 ± 14.3
Diagnosis	
Esophageal malignancy	3
Gastric malignancy	10
Hepatobiliary malignancy	6
Colonic malignancy	36
Other	1
Group 3 intensive care unit anergic patients (n = 21)	
Men	11
Women	10
Mean age (± SD) (yr)	63.9 ± 15.9
Diagnosis	
Sepsis	11
Multiple trauma	10

regulatory cytokine TGF-β did not require prior injection (Table 9). Four hours after injection of antigen, mRNA for IL-8 was detected, regardless of its ability to elicit a DTH reaction in the subject. mRNA for the lymphokines IL-3 and IFN-γ was detected late in the response and only in response to specific antigen (data on irrelevant antigen injections on file). Skin from anergic patients disclosed an identical pattern of mRNA expression, with a lack of mRNA expression for IL-3 and IFN-γ (Table 9).

In addition to recruited cells, normal uninjected skin also contains a small number of memory T cells, which are thought to recirculate through the skin. We monitored such cells by PCR using primers for the epsilon chain of the CD3 molecule. We detected the presence of T cells in normal, uninjected skin derived from DTH-reactive subjects in 15 of 16 instances. Similar analysis at

Table 7. THE EFFECT OF NUTRITIONAL MANIPULATION IN PREOPERATIVE ANERGIC PATIENTS IN GROUP 1

Variable	Regular AA	Enriched AA
Sex		
Men	5	4
Women	3	3
Age (yr)	68.0 ± 7.8	70.1 ± 9.0
APACHE II	7.0 ± 2.3	6.5 ± 2.4
Skin tests		
Day 0		
Anergic	9	6
Reactive	0	0
Day 14		
Anergic	8	4
Reactive	1	2
PHA (cpm × 10^{-3})		
Day 0	13.6 ± 9.0	14.8 ± 8.0
Day 14	17.2 ± 9.5	21.4 ± 9.0
CD3 (Pan T-cells, %)		
Day 0	70 ± 5	69 ± 10
Day 14	66 ± 9	65 ± 8
CD4 (T-helper, % Ts)		
Day 0	43 ± 14	38 ± 10
Day 14	48 ± 12	45 ± 10
CD8 (T-suppressor, % Ts)		
Day 0	21 ± 12	21 ± 10
Day 14	21 ± 9	20 ± 8
Macrophages (% WBC)		
Day 0	7.1 ± 4.0	6.5 ± 4.4
Day 14	7.8 ± 3.0	7.0 ± 2.0
PMN (% WBC)		
Day 0	73.4 ± 10.0	82.0 ± 8.0
Day 14	78.0 ± 9.0	80.0 ± 7.0
PMN adherence (%)		
Day 0	63.5 ± 5.0	62.3 ± 7.0
Day 14	64.6 ± 4.0	62.0 ± 8.0
PMN chemotaxis (cells/hpf)		
Day 0	24.9 ± 8.0	24.0 ± 7.0
Day 14	23.0 ± 3.0	26.0 ± 9.0
C3 (g/L)		
Day 0	0.9 ± 0.4	0.6 ± 0.3
Day 14	1.4 ± 1.4	0.7 ± 0.2
IgG (g/L)		
Day 0	11.5 ± 6.0	11.0 ± 8.0
Day 14	12.8 ± 9.0	10.8 ± 7.0
IgA (g/L)		
Day 0	2.8 ± 1.2	2.9 ± 2.0
Day 14	2.9 ± 1.0	3.1 ± 2.2
IgM (g/L)		
Day 0	1.8 ± 0.5	1.6 ± 1.0
Day 14	1.5 ± 0.9	1.9 ± 1.2
α1-Antitrypsin (g/L)		
Day 0	2.4 ± 1.0	2.8 ± 0.8
Day 14	2.5 ± 1.2	3.0 ± 1.1
α2-Macroglobulin (g/L)		
Day 0	2.1 ± 1.0	2.7 ± 1.2
Day 14	2.5 ± 1.0	2.5 ± 0.2

(continues)

Table 7 (continued). THE EFFECT OF NUTRITIONAL MANIPULATION IN PREOPERATIVE ANERGIC PATIENTS IN GROUP 1

Variable	Regular AA	Enriched AA
CRP (mg/L)		
Day 0	20.4 ± 5.0	24.8 ± 6.0
Day 14	21.2 ± 9.0	22.3 ± 7.0
Albumin (g/L)		
Day 0	32.2 ± 8.0	31.2 ± 8.0
Day 14	35.5 ± 9.0	32.0 ± 7.0
Prealbumin (g/L)		
Day 0	0.20 ± 0.06	0.23 ± 0.04
Day 14	0.21 ± 0.04	0.24 ± 0.02
Transferrin (g/L)		
Day 0	2.1 ± 0.6	2.2 ± 0.4
Day 14	1.7 ± 0.2	1.8 ± 0.3
Retinol Binding		
Day 0	0.04 ± 0.01	0.04 ± 0.01
Day 14	0.04 ± 0.01	0.05 ± 0.01

AA = amino acids; PHA = phytohemagglutinin antigen; PMN = polymorphonuclear neutrophil leukocytes; WBC = white blood cells; CRP = C-reactive protein.
Values are mean ± SD.
There were no statistically significant differences between regular AA and enriched AA.

elective surgical patients without prior infection as well as to those ill enough to require admission to an ICU. We attributed the recent reduction in mortality of anergic patients, as compared to 20 years ago, to improvements in the care of patients, which has eliminated anergy as

Table 8. IN VIVO IGG ANTIBODY RESPONSE TO VACCINATION WITH TETANUS TOXOID OR PNEUMOVAX EXPRESSED AS A RATIO OF DAY 14 LEVEL/DAY 0 LEVEL

Subjects	Tetanus Toxoid (protein Ag)		Pneumovax (polysaccharide Ag)	
	n	Mean ± SEM	n	Mean ± SEM
Healthy controls	26	28.34 ± 0.19	8	4.89 ± 0.08
Electric reactive	11	20.39 ± 0.29*	5	12.17 ± 0.22‡
Elective anergic	9	5.50 ± 0.34*·†	7	7.08 ± 0.24
SICU reactive	8	3.50 ± 0.24*·†	—	ND
SICU anergic	12	1.28 ± 0.17*·†	—	ND

ND = not done.
* p < 0.05 vs. healthy controls (Wilcoxon rank sum test).
† p < 0.05 vs. reactive patients (Wilcoxon rank sum test).
‡ Not significant vs. controls or anergic patients (Wilcoxon rank sum test).

Table 9. ANALYSIS OF SKIN BIOPSIES FROM DTH-REACTIVE AND ANERGIC PATIENTS BY THE POLYMERASE CHAIN REACTION

Cytokine mRNA	Uninjected Skin		4 hr After Injection of Antigen		24 hr After Injection of Antigen	
	Reactive	Anergic	Reactive	Anergic	Reactive	Anergic
TNF-α†	15/15*	6/6	5/6	6/6	2/2	6/6
IL-1β†	16/17	4/6	24/26	15/19	2/2	4/4
TGF-β†	8/12	3/3	5/5	ND	2/2	ND
IL-8‡	0/5	0/4	17/22	7/19	ND	ND
IL-3§	0/4	0/1	1/5	0/1	11/16	0/8
IFN-γ§	0/4	0/1	0/8	0/1	13/16	0/8

TNF = tumor necrosis factor; IL = interleukin; DTH = delayed-type hypersensitivity; TGF = transforming growth factor; ND = not done; IFN = interferon.
* Cytokine mRNA detected per biopsy analyzed.
† Not significant for all comparisons.
‡ p < 0.05 uninjected skin vs. 4 hr postantigen injection for reactive and anergic patients.
§ p < 0.05 at 24 hr after injection of antigen, reactive vs. anergic patients.

a contributing factor for increased mortality in elective surgery. Improved preoperative, intraoperative, and postoperative care has reduced the level of bacterial infectious challenge after surgery, making immune defects signaled by anergy irrelevant. This is similar to the treatment of cases of severe combined immunodeficiency, in which patients are placed in a pathogen-free environment (The Boy in the Bubble).

Similar reductions in mortality have not occurred in the surgical ICU. Severe trauma or major surgical complications require invasive monitoring devices. Surgery often fails to completely eliminate the source of bacterial contamination. This persistent "bacterial challenge," along with the immune alterations signaled by anergy and changes in nonspecific host defense detected in all surgical ICU patients, continues to render such patients susceptible to infection-related mortality.

Unless severe malnutrition is present, parenteral nutritional manipulation of the immune response will not have a major effect on outcome, because it fails to influence host immune functions, as has been demonstrated in this and other reports.[19] Enteral feeding formulations have shown some benefit.[20]

The greatest mortality rate was observed among anergic patients who developed or had subsequently developed a serious infection. This indicates that such patients cannot contain an infection, thus the infection spreads to vital organs, causing death, or that these patients react inappropriately to the infectious challenge systematically and die of multiple organ dysfunction. This issue will only be resolved when we have a full understanding of how infection kills the patient. We did not find major biochemical or clinical comorbid factors among anergic patients that might make them more susceptible to an

infectious challenge and thus explain their increased mortality. The data we have accumulated over the last 20 years suggest that anergic patients should not be so susceptible to bacterial infections. Nonspecific host defense, as measured by polymorphonuclear neutrophil function, does not differ among DTH-reactive and anergic patients. No correlation was found between skin-test response and PMN function, although we have found a strong correlation between an activation event, such as trauma or infection, and altered PMN function.[21] The detailed analysis reported in the current study shows that PMN function is similar among anergic and reactive patients. The antibody response to a protein antigen, tetanus toxoid, which is T-cell dependent,[22] was reduced in anergic patients, but the antibody response to a polysaccharide antigen, Pneumovax, which is less dependent on T cells,[23] was normal. Because most bacterial antigens are polysaccharides, the immune defect that puts the anergic patient at risk is not likely to be the antibody response. The explanation of the difference in antibody responses to protein and polysaccharide antigens may be related to T-lymphocyte function.

Because the bacterial burden in anergic surgical patients did not consist of intracellular bacteria, we did not attribute increased mortality among these patients to a lack of DTH reactivity per se. However, the DTH reaction and most if not all other forms of immune reactivity depend on T-lymphocyte function and T-lymphocyte traffic, as is demonstrated in lymphocyte recirculation or the homing to sites of inflammation. More likely, a lack of DTH reactivity among surgical patients represents a broader immune deficit in T-lymphocyte function or traffic. To investigate T-lymphocyte function in anergy, we initially used an in vitro correlate of DTH, that is,
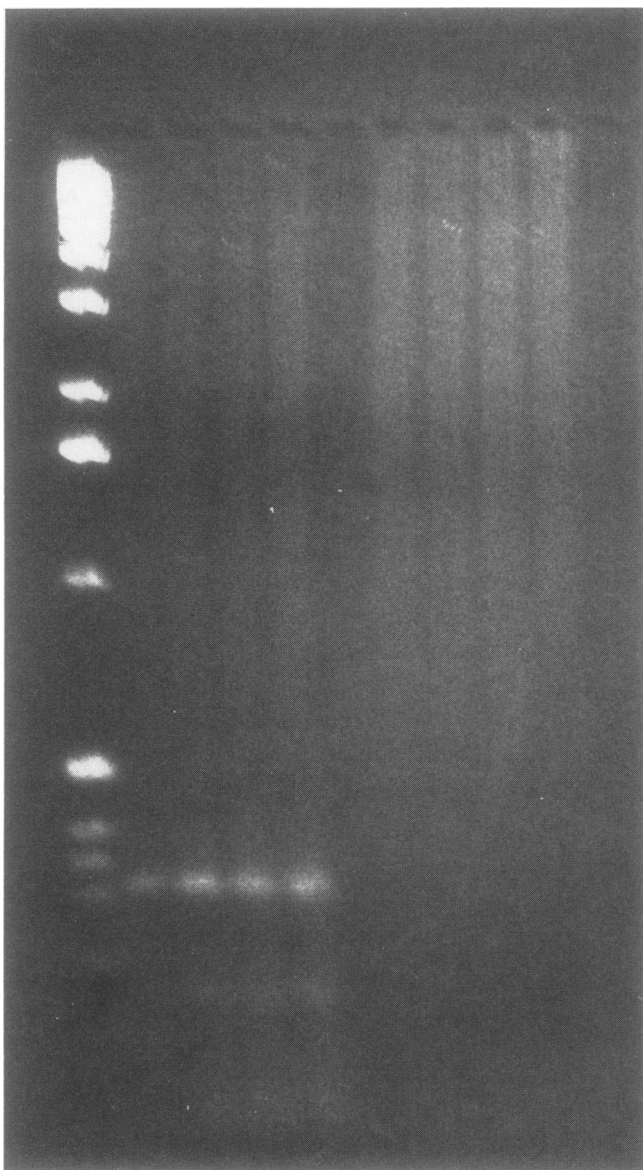
Figure 3. Agarose gel electrophoretogram of PCR products from skin biopsies of (left quartet) 4 DTH-reactive patients and (right quartet, negative) 4 anergic patients by means of CD3 primer and amplification of 30, 35, 40, and 45 cycles. DNA molecular-weight markers are shown at left.

cell proliferation in response to antigen. We found that peripheral blood lymphocytes from anergic patients could respond fully to alloantigens as well as to some of the same antigens to which these patients did not react to in their skin.[24] This observation provided the hallmark of immune dysfunction in anergy; memory T cells were present in blood but were either absent or could not respond to antigen in skin. Assuming that the function of T cells in DTH is to produce lymphokines and that recruitment of cells to the DTH site requires the presence of inflammatory cytokines, we explored the availability of cytokines in anergic skin. In the first instance, we gen-

erated cytokines from healthy donors in mixed leukocyte cultures or by incubation of peripheral blood lymphocytes from anergic patients (reactive *in vitro* with purified protein derivative) with purified protein derivative and injected these supernatants alone or together with purified protein derivative in an attempt to elicit a DTH reaction. We found that supernatants containing cytokines (but not those from heat-killed cells or from cells incubated without antigen) could restore the DTH reaction in the majority of anergic patients when injected together with antigen. Injection of the antigen alone or of the culture supernatant alone was ineffective.[25] We obtained identical results using skin chambers placed over tape-stripped skin,[26] which allowed us to quantitate the DTH reaction by enumerating the infiltrating mononuclear cells. We found an over 100-fold difference between the number of cells recovered from DTH sites from anergic compared with reactive patients, in which the number of cells in the former were fully restored by co-injection of antigen and cytokines.[27] In contrast, injection of the cytokines and antigen into the skin of DTH-reactive patients merely doubled the number of recovered cells. We therefore believe that a deficit may exist in the production of one or several cytokines in anergic skin.

The only statistically significant difference detected in these experiments between the reactive and anergic groups was a lack of mRNA expression of IL-3 and IFN-γ in the skin of anergic patients. Because up-regulation of mRNA for these two cytokines in the DTH-reactive group was antigen specific, we attributed them to lymphocytes in the skin. Because we detected IL-3 and IFN-γ only at 24 hours into the reaction, when the DTH site was infiltrated by blood-derived lymphocytes, we further attributed them to the infiltrating cells. Because there is no lymphocyte recruitment to the injection site in anergic patients, this deficit in anergic skin was expected and is compatible with the interpretation that their source in DTH-reactive individuals is the recruited lymphocyte.

In addition to recruited cells, the normal uninjected skin also contains a small number of memory T cells,[28,29] which are thought to recirculate through the skin.[30] In our investigation of the DTH reaction, the only deficit we found in uninjected anergic skin was the lack of detectable T cells by PCR. To attribute the lack of DTH reactivity to this absence requires that such cells play an obligatory role in the normal DTH reaction, which has not been demonstrated. Recent experiments performed in our laboratory (M. Hassan Zahrae, J. Gordon, unpublished data, 1995) have shown that such cells can respond to antigen by a short burst of IFN-γ synthesis, which is maximal at 2.5 hours and which wanes by 4 hours after activation (this early and narrow time frame may explain why we failed to detect mRNA for IFN-γ in skin from reactive patients analyzed 4 hours after injec-

Table 10. THE ANALYSIS OF CIRCULATING AND EXUDATE PMN FUNCTIONS IN 36 ELECTIVE SURGICAL PATIENTS

Patient Group	PMN Exudation (PMNs/site)	CD11b (mcn)	FMLP-r (mcn)	CD14 (mcn)	CD16 (mcn)	Superoxide (mcn)	Candidcidal Activity (% killing)
Circulating PMNs							
Reactive (n = 20)	—	268 ± 173	32 ± 9	7 ± 3	43 ± 23	2.9 ± 1.2	10.8 ± 4.6
Anergic (n = 16)	—	450 ± 382	35 ± 9	6 ± 3	42 ± 16	2.9 ± 1.0	9.1 ± 4.6
Exudate PMNs							
Reactive (n = 20)	$2.2 ± 0.5 × 10^6$	2430 ± 374	61 ± 15	24 ± 8	97 ± 63	8.3 ± 1.8	18.7 ± 5.5
Anergic (n = 16)	$2.6 ± 1.2 × 10^6$	2302 ± 462	63 ± 14	24 ± 7	122 ± 53	6.4 ± 2.2	14.8 ± 7.3

CD11b = CD11b adhesion molecule; FMLP-r = formyl methyl leucine phenylalanine receptor; CD14 = CD14 receptor (lipopolysaccharide receptor); CD16 = opsonization receptor; mcn = mean channel number; PMNs = polymorphonuclear neutrophil leukocytes.

tion of antigens). Interferon-gamma has been shown to be a key mediator in the skin to up-regulate adhesion receptors on skin vascular endothelium, which facilitates the influx of leukocytes[31] and orchestrates the activation of the skin immune system.[32] Interferon-gamma is made only by natural killer cells, which are absent from skin, and by lymphocytes. Accordingly, this cytokine made by skin-resident T cells in response to antigen may mediate this pivotal function in the initiation of the DTH reaction, and its absence could constitute the immune deficit in anergy.

If the foregoing interpretation is correct, then the absence of DTH reactivity in anergic patients may not only signal a general immune dysfunction, but also indicate a defective host defense mechanism, at least at the level of the skin. Extending the defective T-cell homing seen in the skin to other tissues in which subsets of memory T cells recirculate, such as the lymphoid tissues of the gastrointestinal tract, may explain the increased septic-related mortality seen in our patients. A key component of the inflammatory reaction may not be initiated at the two major sites of potential entry of pathogens in surgical patients: the skin and the gut. This could allow for a bacterial infection to establish itself , spread, and produce the systemic alterations of multiple organ dysfunction and potential death of the patient. Further work in this area focusing on surgical ICU patients, in whom anergy still indicates decreased survival probability, is indicated.

Acknowledgments

References

1. MacLean LD, Meakins JL, Taguchi K, et al. Host resistance in sepsis and trauma. Ann Surg 1975; 182:207–211.
2. Johnson WC, Ulrich F, Meguid MM. Role of delayed hypersensitivity in predicting postoperative morbidity and mortality. Am J Surg 1979; 137:536–539.
3. Adami GF, Terrizzi A, Vita M. The assessment of skin tests in surgery. Surg Italy 1980; 10:297–301.
4. Tasseau F, Gaucher L, Nicolas F. Cell-mediated immunity studied by skin tests in patients receiving intensive care: prognostic value of repeated tests. Study of some factors predisposing towards anergy. Semin Hop Paris 1982; 58:781–786.
5. Kienlen J, Chardon P, Nury G, et al. Study of delayed skin hypersensitivity using the multitest in an intensive care unit. Ann Anesthesiol Fr 1981; 22:285–291.
6. Brown R, Bancewicz J, Hamid J, et al. Failure of delayed hypersensitivity skin testing to predict post-operative sepsis and mortality. Br J Surg 1982; 284:851–853.
7. Gertner MH, Mullen JL, Buzby GP, et al. Implications of malnutrition in the surgical patient. Arch Surg 1979; 114:121–125.
8. Harvey KB, Moldawer LL, Bistrian BR, Blackburn GL. Biological measures for the formulation of a hospital prognostic index. Am J Clin Nutr 1981; 34:2013–2022.
9. Morath MA, Miller SF, Finley RK. Nutritional indicators of post-burn bacteremic sepsis. JPEN 1981; 5:488–491.
10. Casey J, Flinn WR, Yao JS, et al. Correlation of immune and nutritional status with wound complications in patients undergoing vascular operations. Surgery 1983; 93:822–827.
11. Hoffer EC, Meador CK, Simpson DC. A relationship between whole body impedance and total body water volume. Ann NY Acad Sci 1970; 110:452–461.
12. Rode HN, Christou NV, Bubenik O, et al. Lymphocyte function in anergic patients. Clin Exp Immunol 1981; 47:155–159.
13. Christou NV, Meakins JL. Neutrophil function in anergic surgical patients: Neutrophil adherence and chemotaxis. Ann Surg 1979; 190:557–564.

14. Nohr CW, Christou NV, Rode H, et al. *In vivo* and *in vitro* humoral immunity in surgical patients. Ann Surg 1984; 200:373–380.

15. Dallman MJ, Montgomery RA, Larsen CP, et al. Cytokine gene expression: analysis using northern blotting, polymerase chain reaction and *in situ* hybridization. Immunol Rev 1991; 119:163–168.

16. Leung JCK, Lai CKW, Chui YL, et al. Characterization of cytokine gene expression in CD4+ and CD8+ T cells after activation with phorbol myristate acetate and phytohaemagglutinin. Clin Exp Immunol 1992; 90:147–152.

17. Zimmerli W, Seligman B, Gallin JI. Exudation primes human and guinea pig neutrophils for subsequent responsiveness to the chemotactic peptide N-formylmethionylleucylphenylalanine and increases complement component C3bi receptor expression. J Clin Invest 1986; 77:925–933.

18. Yee J, Giannas B, Kapadia B, et al. Exudative neutrophils: modulation of microbicidal function in the inflammatory microenvironment. Arch Surg 1994; 129:99–105.

19. Buzby GP. Overview of randomized clinical trials of total parenteral nutrition in malnourished surgical patients. World J Surg 1993; 17:173–177.

20. Moore FA, Moore EE, Brown RO. Clinical benefits of an immune-enhancing diet for early postinjury enteral feeding. J Trauma 1994; 37:607–615.

21. Christou NV, Tellado-Rodriguez JM. *In vitro* polymorphonuclear function in surgical patients does not correlate with anergy but with "activating" processes such as sepsis or trauma. Surgery 1989; 106:718–724.

22. Baker PJ, Amsbaugh DF, Stashak PW, et al. Regulation of the antibody response to pneumococcal polysaccharide by thymus-derived cells. Rev Infect Dis 1981; 3:332–341.

23. Davies AJS, Carter RL, Leuchars E, et al. The morphology of immune reactions in normal, thymectomized and reconstituted mice. III. Response to bacterial antigens, salmonella flagellar antigen and pneumococcal polysaccharide. Immunology 1970; 19:945–957.

24. Rode HN, Christou NV, Bubenik O, et al. Lymphocyte function in anergic patients. Clin Exp Immunol 1981; 47:155–159.

25. Rode HN, Puyana JC, MacPhee M, et al. Role of cytokines in the restoration of the delayed-type hypersensitivity reaction of anergic patients. Clin Exp Immunol 1988; 71:159–163.

26. MacPhee JJ, Rode H. Broadhead M, et al. Skin window chambers, a novel method for recovering the cells involved in delayed-type hypersensitivity. J Immunol Method 1987; 103:267–273.

27. MacPhee M, Christou NV, Gordon J, et al. Quantitative and qualitative study of the restoration by cytokines of mononuclear cell delivery to skin test sites in anergic surgical patients. Arch Surg 1988; 123:1470–1473.

28. Bos JD, Zonneveld I, Das PR, et al. The skin immune system (SIS): Distribution and immunophenotype of lymphocyte subpopulations in normal human skin. Invest Dermatol 1987; 88:569–572.

29. Foster CA, Yokozeki H, Rappersberger K, et al. J Exp Med 1990; 171:997–999.

30. Mackay CR, Nature 1991; 349:737–739.

31. Barker JN, Allen MH, MacDonald DM. The effect of *in vivo* interferon-gamma on the distribution of LFA-1 and ICAM-1 in normal human skin. J Invest Dermatol 1989; 93:439–444.

32. Nickoloff BJ. The role of gamma interferon in epidermal trafficking of lymphocytes with emphasis on molecular and cellular adhesion events. Arch Dermatol 1988; 124:1835–1842.

Discussion

DR. DAVID L. DUNN (Minneapolis, Minnesota): This study represents the culmination of two decades of research focusing on a specific question: Why do surgical patients develop an infection, how can we best identify this, and how can we prevent it?

Over the years, Drs. Christou, Meakins, and MacLean have provided surgical science with a wealth of data in this regard, the interpretation of which may have significant clinical ramifications.

From the current study, we now know that the phenomenon of anergy represents a defect in T-lymphocyte trafficking and, therefore, T-lymphocyte activity in the tissue compartment. This is clearly something that we suspected, but it has now been identified with the current *in vitro* and *in vivo* data.

The authors chose the skin as an easy window to view this phenomenon and infer similar T-cell deficiency in tissues throughout the body. Perhaps not surprisingly, cytokine message in the form of interleukin-3 and interferon-gamma mRNA was not present in the skin of anergic patients because recruited T cells, as Dr. Christou pointed out, were not there.

The importance of this finding, however, I think was somewhat downplayed in both the presentation and the manuscript, which really contains a wealth of data and which I recommend to you. And the importance of the finding is that we really now have a mechanism to explain the phenomenon of anergy. It is very clear-cut what is occurring in these patients.

The clinical findings of this study, however, were mixed. The good news is that so-called walk-in anergic patients do well, probably based on incremental improvements in medical and surgical care that have occurred over this two-decade period. The bad news is that anergy in the surgical intensive care unit patient continues to be associated with approximately a 30% mortality rate. And I would point out to you that this mortality rate demonstrated by complete anergy is very similar if not identical to that of current studies in which sepsis syndrome was studied. I have several questions for the authors.

First, in the intensive care unit, does their study really tell us whether anergy is associated with or a consequence of infection? As Dr. Christou pointed out, this is probably the key question. But my question for him revolves around whether or not low-grade infection was present in some patients.

He clearly demonstrated that this was not the case with regard to bacteremia and intra-abdominal infection. But I think we need to ask the question as to whether or less severe infections concurrently were present. This is an extremely hard question to answer without performance of a large prospective study in which infection surveillance at numerous sites takes place. However, I would appreciate hearing the authors' comments along these lines.

Also, this study brings into clear focus the importance of T cells and the composite immune response to infection. I firmly believe that T cells and macrophages exert numerous effects by directional signaling, probably through cytokine release, an activity that is extremely important in phagocytic recruitment and enhancement of activity of phagocytic cells at the site of infection.

Dr. Christou, please hypothesize how the various components of the immune system function in normal and anergic patients. Is the T-cell portion of the response redundant in most patients, except for those that are anergic? Or is there a spec-