Systemic Antibacterial Mechanisms in Trauma

Lloyd D. MacLean, M.D., Ph.D.

Department of Surgery, Royal Victoria Hospital and McGill University, Montreal, Canada

Host defense mechanisms are cell-mediated, humoral and phagocytic. Acquired derangements of one or many of the immune antibacterial defenses are common after injury. These defects include (a) a failure of T-cell response (DTH) to antigen stimulation manifested by negative skin test reactions to common antigens (anergy); (b) a failure of humoral response, or B-cell depression after appropriate antigen stimulation; and (c) development of serum inhibitors of phagocyte chemotaxis.

Anergy reflects an immunodeficiency state more profound than impaired T-cell function alone and, if left uncorrected, is associated with a markedly increased incidence of sepsis and associated mortality. Therapeutic implications of these findings for now and the future are summarized.

The host defense mechanisms against bacterial invasion are humoral, cell-mediated, and phagocytic. Each protective element functions in an integrated fashion with the others to control and kill the invading organism(s). Surgeons are concerned with alterations in these normal functions because sepsis is the principal cause of morbidity and mortality in patients following trauma and major surgery. Abnormalities in the normal protective mechanisms can now be measured in patients, and can serve to warn of developing sepsis and the need for further different treatment. It is exciting to think that infection in surgical patients might be eliminated by prophylactic techniques just as smallpox has been eliminated by vaccination.

The Inflammatory Response

Inflammation with its signs—rubor, calor, dolor, and tumor—is the normal local reaction of vascularized tissue to injury.

There is initially a vascular response which is mainly vasodilation (heat and redness), accompanied by an increased permeability of vessels 20–100 μm in diameter, and a slowing of blood flow in these vessels due to the increased viscosity of packed red cells. These changes permit increased adherence to the endothelial lining by polymorphonuclear leukocytes (PMN) and diapedesis or emigration into the inflammatory focus. During the first 24 hours after injury or focal infection, there is also an increase in interstitial osmolarity, accumulation of protein (swelling), and generation of chemotactic factors.

Chemotactic factors can be generated by (a) clotting of blood (kallikrein, plasminogin activator, fibrinopeptides); (b) injured tissues (collagen fragments); (c) complement (C 5a is the most active and important of complement-derived components); (d) bacteria (liberation of bacterial peptides); (e) lymphocytes (lymphokines derived from antigen-stimulated lymphocytes); and (f) prostaglandins (causing the pain of inflammation).

A number of substances present in serum and lymphocyte granules also serve to inhibit the chemotactic response. These inhibitors may prevent excessive activation of chemotactic mechanisms and may also retain the leukocytes in an area of inflammation once attracted there. Another mechanism to retain leukocytes is the refractoriness of leukocytes to respond further to chemotactic agents of the same or different type. Additionally, many bacterial and viral toxins can inhibit leukocyte locomotion.

Disposal of most bacteria and fungi is accomplished by phagocytosis, which is the single most

important process in control of infection. Following release from the marrow, only about half of the intravascular granulocytes circulate; the remainder are sequestered in small blood vessels or become adherent to the endothelium of larger vessels. These may be released after administration of catecholamines or corticosteroids and may account for the transient neutrophilia that follows stress or hypovolemic shock. In acute inflammatory conditions, neutrophils migrate into tissues within a few hours. The simplest method for studying chemotaxis in vivo is the skin-window technique of Rebuck and Crowley [1]. A sterile glass coverslip or cup is placed over a standard skin abrasion and is periodically removed or washed through with Hanks' solution and the cells are counted and identified. Neutrophils move rapidly and are present in large numbers by 4 hours; monocytes migrate more slowly and become the prominent cell type within 12 to 24 hours. This is a pattern typical of surgical wounds.

Phagocytosis and the release of powerful enzymes by neutrophils and macrophages comprise 2 of the major benefits derived from the accumulation of leukocytes at the site of injury. These phagocytes will, on occasion, recognize and engulf bacteria or extraneous matter (e.g., latex beads) in the absence of serum, but most microorganisms are not attacked until they are coated by naturally occurring serum factors (opsonins). Two well-known opsonins are IgG especially from hyperimmune individuals and the opsonic fragment of complement (C3). Engulfment of the particle follows opsonization and ultimately intracellular killing by both oxygen-dependent as well as oxygen-independent mechanisms.

The host phagocytic system can be visualized as operating in 3 areas. The first is host defense at the site of injury, already described. If bacterial invasion is not contained in the local area of injury, passage into the lymphatic network occurs. Lymph node macrophages provide an effective barrier to the dissemination of bacteria into the bloodstream.

When the bloodstream is invaded, the reticuloendothelial cells that line the vascular compartment, particularly in the liver, spleen, and bone marrow, provide the third echelon of phagocytic defense. The Kupffer cells of the liver are especially efficient in a clearance role because 25–30% of the cardiac output passes by these cells every minute.

While the major opsonic proteins for bacterial clearance are immunoglobulins and the C3b and C5b components of activated complement, the major opsonic protein for non-bacterial particulate matter is opsonic α 2-surface binding (SB) glycoprotein, which is identical to cold-insoluble globulin or plasma fibronectin. The normal function of the reticuloendothelial system (RES) is important in

trauma because it has been demonstrated that (a) colloid-induced RES blockade will decrease resistance to trauma and shock, (b) RES stimulation will increase resistance to experimental trauma and shock, and (c) deterioration of hepatic or splenic reticuloendothelial phagocytic clearance capacity occurs following trauma and shock and is usually proportional to the degree of injury. Furthermore, if opsonic fibronectin is depleted, liver reticuloendothelial clearance capacity falls, and during this time animals have increased sensitivity to shock and trauma as well as to bacterial sepsis. Depression of RES function with depressed phagocyte clearance of bloodborne particles usually results in increased localization, especially in lung and kidneys, with resulting impaired function of these organs [2, 3].

Cell-Mediated Immunity

Many microbes, especially those that live within host cells, cannot be eliminated effectively by opsonization and phagocytosis. Host resistance to these microbial agents depends upon specifically sensitized thymus-derived (T) lymphocytes. Cell-mediated immunity refers to this system, even though immune cells (e.g., antibody-manufacturing B cells) and nonimmune cells (e.g., phagocytes of all types) participate in all aspects of host defense. Figure 1 illustrates the complex interaction of cell types for maximum lymphocytic proliferative activity and the differentiation of the cells into mature effector T cells or antibody-producing B cells.

Anergy

The normal skin test response expected when a common antigen to which the patient has likely been exposed in the past appears in Figure 2. Macrophage-processed antigen is necessary to sensitize the T cell. Exposure to this antigen at a later date causes a reaction due to the release of lymphokines with vascular changes and accumulation of cells in the local area which can be palpated at 24–48 hours. A nodule of 5 mm or greater at 24–48 hours is considered a normal response. A patient who shows no response at these times when 5 antigens are used is referred to as *anergic*, and a single response out of 5 is called *relative anergy* (Table 1).

Defects in Host Defense

Primary and continuous immunodeficiencies include: (a) antibody or B-cell deficiency, (b) cellular or T-cell deficiency, (c) phagocytic cell dysfunc-
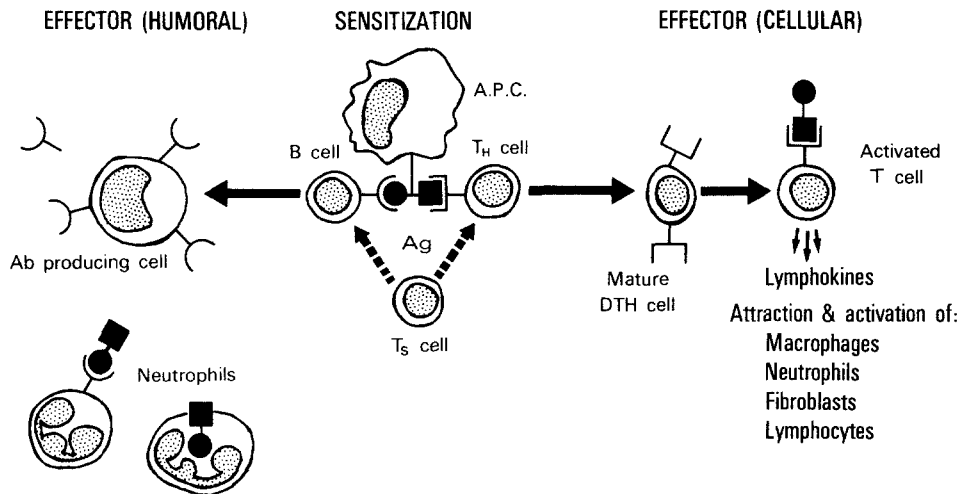
Fig. 1. Cell interactions in immune responses. The antigen presenting cell (APC), frequently a macrophage, offers antigen to either T_H (helper) cell or a DTH precursor cell which in turn becomes a mature DTH cell capable of reacting if the antigen is again encountered. Cellular effector mechanisms are pictured on the right of the figure. The humoral effector mechanisms are on the left with antibody production and engulfment of antigen by polymorphonuclear neutrophils. T_S is a suppressor T cell.

tion, (d) disorders of complement, and (e) combined immunodeficiency disorders (T and B cell).

Acquired immunodeficiencies are much more common but less precisely defined. Unlike congenital defects in which a single defect frequently accounts for the clinical manifestations, acquired defects often involve more than one component of host defense.

In clinical practice, there are a number of situations in which one could predict an increased probability of sepsis—advanced age, major operation with contamination, trauma, diabetes, uremia, cancer, poor nutrition, severe pancreatitis, and hemorrhagic shock. Not all patients in these high-risk groups develop abscess formation and septicemia, but identification of those likely to is an important clinical task and should be instituted before, rather than after, the onset of infection. Using the recall skin-test antigen test (Table 2) and defining sepsis as a positive blood culture or an abscess identified at surgery or autopsy, it has been shown that patients who are anergic (A) and relatively anergic (RA) have a significantly increased incidence of sepsis and death as compared to those whose skin-tests remain normal (N). These results have been confirmed and show that skin-testing preoperatively identified a group of patients at risk for increased sepsis and mortality following surgery. Sequential skin-testing indicates that patients who were anergic and recover normal responses have a higher incidence of sepsis while anergic, but have a much improved mortality rate compared to those who remain anergic [4].

Cutaneous responses to recall antigens—delayed hypersensitivity reactions (DTH)—are classically considered to be a reflection of cell-mediated immunity. The infections for which cell-mediated immunity is effective are rare in surgical patients, and the

organisms that produce sepsis in anergic patients are usually common bacteria. Host response against these organisms is mediated by the humoral and phagocytic components of host defense. This suggests that even though the delayed hypersensitivity reaction is altered and correlates with increased rate and severity of infections, the defect may be a general one and other anti-infective aspects of host defense are likely to be abnormal.

Lymphocyte Function in Anergic Patients

In vitro lymphocyte responses are normal in most instances in anergic patients, in that the standardized mixed leukocyte culture reaction elicits responses within the normal range in over 80% of tests [5]. Similarly, a positive in vitro proliferative response is produced by PPD with lymphocytes from anergic patients in the same numbers as in PPD reactive patients in a skin-tested hospital population. The ability of in vitro activated lymphocytes to elicit a skin reaction when cultured with PPD and then reinjected intradermally back into the same anergic patients suggests that one of the acquired immune defects in anergic patients is an in vivo block of lymphocyte activation. If the cells are not activated in vitro or not stimulated by PPD, a skin reaction is rarely obtained [5].

Dysfunction of Humoral Immunity in Anergic Surgical Patients

The "anergic environment" that inhibits normal lymphocyte (T) cell response also extends to B-cell or humoral immunity [6]. This hypothesis was tested in 5 healthy control individuals and 14 anergic
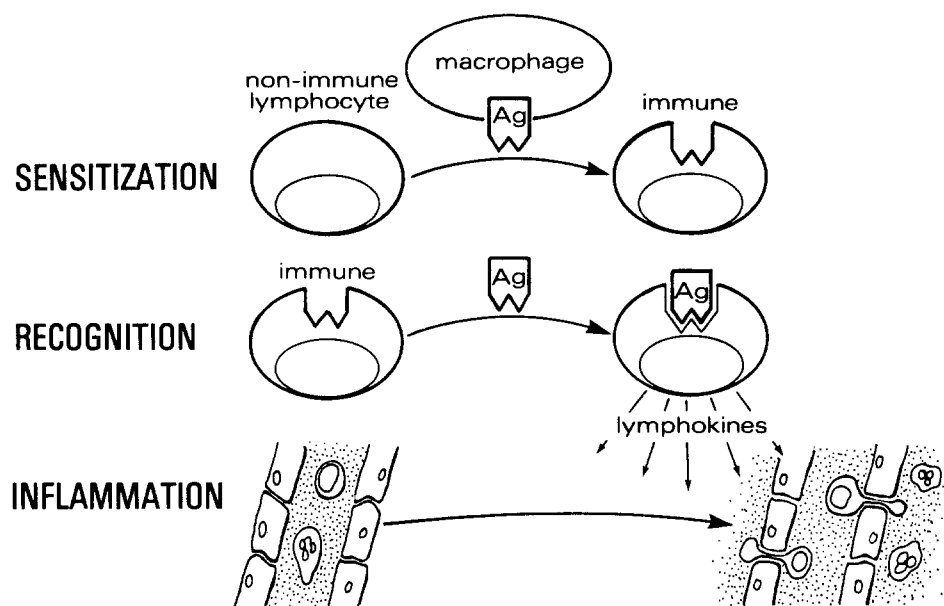
Fig. 2. The DTH response (anergy test) as produced by skin antigens to which a patient has previously been exposed. The release of lymphokines and accumulation of cells in the area can be palpated. It indicates that recognition and activation have occurred. In anergic patients, the cells are capable of reacting in vitro when stimulated with antigen, suggesting that there is an in vivo inhibition of the activation stage of the reaction, i.e., the immune lymphocyte does not release lymphokines when antigen is presented intracutaneously.

patients by the measurement of antibody response to the injection of tetanus toxoid (TT). Whereas 4 of the 5 healthy controls produced specific anti-TT antibodies in their plasma, immunization of the 14 anergic patients elicited no antibody response. Accordingly, the lack of DTH responsiveness in anergic patients may be seen as an indicator of other immune disorders, which in turn could explain the high incidence and fatal outcome of infections usually combated primarily by a humoral type response. These results also suggest that if lack of an antibody response is responsible for the high risk of infections, then passive immunization with specific antibodies directed against the common infectious agents encountered may help reduce the risk and protect anergic patients during the critical period of recovery from trauma or surgery.

Phagocytic Cell Function

Injured patients demonstrate abnormal polymorphonuclear neutrophil (PMN) function within 2 hours of injury [7]. This is expressed as depressed chemotaxis and abnormal adherence and appears to be related to circulating inhibitors [7]. The severity of the injury is correlated with the duration of depressed host defense. No gross abnormalities of serum immunoglobulins or complement levels are detected early after injury when PMN function is depressed. It is unlikely that malnutrition is involved either, since many injuries occur in young, healthy and well-nourished individuals who showed abnormal PMN reaction early after injury. An in vivo demonstration of decreased delivery of PMN

to an area of inflammation was also reported by Superina et al. [8]. Skin-window chambers were fashioned by creating epithelial forearm abrasions over which water-tight tissue chambers were taped. Hanks' balanced salt solution (1.5 ml) was placed in the chamber and the cells were allowed to accumulate. The chambers were emptied and refilled with the solution every 2 hours for 24 hours and the cells counted and characterized. Patients with normal skin-test results delivered more PMN to the chambers than did anergic patients. This difference was highly significant at all times tested.

The increased incidence of sepsis in anergic patients may also be the result of a failure of delivery of phagocytic cells to a septic focus in effective numbers. The early lack of a strong cellular response in reaction to a bacterial inoculum in vivo may seriously impair any further effort to contain the septic process. This observation agrees with the concept that an "early decisive period" is crucial in determining the outcome of any bacterial challenge [9].

Can Protein-Sparing Alter Cellular Functions?

Trauma or surgery of sufficient magnitude will produce skin-test anergy in the postoperative or postinjury period. This is a time of vulnerability for these patients and might be related to malnutrition, especially if cellular malfunction persists for over 2 weeks. It has been demonstrated that peripherally administered casein hydrolysate will spare nitrogen losses and preserve body cell mass after major surgery [10].

Table 1. Common skin test antigens.[a]

1. PPD (5 T.U/ml)
2. Mumps (undiluted)
3. Candida (1,000 u/ml)
4. Trichophyton (1,000 u/ml)
5. Varidase (SK/SD) (1,000 u/ml)

[a]Administered as 1/10 ml intracutaneously and read at 24 and 48 hours.

Table 2. Clinical outcome related to least reactive skin test in 1,776 surgical patients.[a]

Skin Test	Patients	Sepsis (%)	Mortality (%)
Normal	1,172	88 (7.5)	25 (2.1)
Relative anergy	178	57 (32)	27 (15)
Anergy	426	200 (47)	149 (35)

[a]From Surgical Infectious Diseases, R. L. Simmons and R.J. Howard, editors, New York, Appleton-Century-Crofts, 1982, by permission of author and publisher.

Utilizing the same technique in a randomized fashion, either glucose only or peripheral amino acid was used to maintain nutrition after major surgery. No difference in immunocompetence occurred between the 2 groups of patients.

There was no difference in the rate of recovery from surgery, nor in the sepsis rate in the 2 groups. There was an equal increase in postoperative PMN adherence in the 2 groups which returned to normal by the 7th postoperative day. Chemotaxis of PMNs was equally depressed, and there was no difference in the clinical outcome. Recovery of immune competence as measured by these methods usually occurs spontaneously and is unaffected by postoperative protein-sparing intravenous fluid [11]. The fact remains that serious infections, including septicemia, are most likely to occur during periods of anergy whether the patient is receiving protein-sparing therapy or not.

Malnutrition can cause defects in host resistance and may be present as an isolated defect which, when corrected, will markedly improve the prognosis of injured patients. This subject is discussed in other sections of this symposium.

Chemotactic Inhibitors

The sera of anergic patients inhibits chemotaxis of PMNs and monocytes in vitro [12]. Chemotaxis returns to normal as skin testing improves. Following major injury, neutrophil chemotaxis is reduced to anergic levels within hours of injury. Recovery can take weeks. Serum from anergic patients con-

sistently inhibits chemotactic responses of cells from normal volunteers. Two inhibitors have been described: one has a molecular weight (MW) of approximately 110,000 and can be identified in all sera; the other, heavier inhibitor (MW = 310,000) was found only in anergic individuals. These studies suggest that the larger inhibitor causes the decreased PMN chemotaxis and as it disappears, chemotaxis improves and skin tests return to normal [7].

Therapeutic Implications

Trauma promotes a myriad of systemic reactions in defense of bacterial infection. The anergic state has been useful as a broad screening technique that indicates inappropriate immune defense at that time in that patient. The first therapeutic requirement is to administer prompt, good surgical care (i.e., debride and close wounds, drain abscesses, and ensure cardiopulmonary stability). These surgical measures alone will reverse anergy in many severely injured patients. If anergy persists over 2 weeks, malnutrition may be a contributing factor and should be treated aggressively by total enteral or parenteral nutrition.

Undrained abscesses are a frequent cause of anergy after injury and the abnormal skin test should alert the surgeon to investigate this possibility. It has now been clearly demonstrated in animals that intraperitoneal sepsis does induce anergy in a dose-related fashion and is associated with a high mortality rate if left undrained [13].

Inhibition of normal chemotaxis and the DTH response is induced by the serum of injured patients. Attempts to reverse this inhibition by immunomodulators such as Levamisol® is a new, unproven but interesting idea. It is known that "walking anergic" patients, i.e., patients admitted for elective surgery who are skin-test negative to 5 recall antigens, have a decreased bactericidal capacity and have serum that will inhibit T-cell function. Furthermore, this inhibition can be reversed by Levamisole® in vitro. This or other immunorestorative therapy is likely to be useful in injured patients in the future.

Active immunization is currently used to control tetanus, diphtheria, pertussis, polio, measles, and rubella in the general population as well as typhoid, typhus, and influenza in selected persons. There is evidence that Pseudomonas heptavalent vaccines can protect burn patients from Pseudomonas infections. It is appropriate that all splenectomized patients should receive the polyvalent pneumococcal vaccine.

Passive immunoprophylaxis is useful for botulism, diphtheria, viral hepatitis, measles, rabies, tetanus, pseudomonas sepsis, and varicella zoster. In most cases, hyperimmune IgG of human origin is available. This technique may be useful for other infections even in traumatized patients.

Résumé

Les défenses de l'organisme chez la traumatisé sont à la fois cellulaires, humorales et phagocytaires. Les altérations de l'une ou de plusieurs des défenses antibactériennes sont communes après tout traumatisme. Elles comprennent: 1) la défaillance de la réponse des cellules T à la stimulation antigénique qui se manifeste par une réaction cutanée négative aux antigènes courants (l'anergie); 2) la défaillance de la réponse humorale ou état défensif des cellules B, après stimulation antigénique appropriée; 3) le developpement des inhibiteurs sériques de la chemotaxis phagocytaire.

L'anergie reflète un état de déficience immunologique plus profond que l'altération isolée des cellules T. Quand elle n'est pas corrigée elle entraîne une élévation du taux de l'infection et de la mortalité. Les implications thérapeutiques liées à ces manifestations sont passées en revue aussi bien pour le temps présent que pour le futur.

References

1. Rebuck, J.W., Crowley, J.H.: Method of studying leukocyte functions in vivo. Ann. N.Y. Acad. Sci. 59:757, 1955
2. Kaplan, J.E., Saba, T.M.: Humoral deficiency and reticuloendothelial depression after traumatic shock. Am. J. Physiol. 230:7, 1976
3. Saba, T.M.: Reticuloendothelial systemic host defense after surgery and traumatic shock. Cir. Shock 2:91, 1975
4. Meakins, J.L.: Alterations of host defenses in surgical patients. In Surgical Infectious Diseases, R.L. Simmons and R.J. Howard, editors, New York, Appleton Century Crofts, 1982, pp. 278
5. Rode, H.N., Christou, N.V., Bubenia, O., et al.: Lymphocyte function in anergic patients. Clin. Exper. Immunol. 47:155, 1982
6. Mazaheri, R., Rode, H.N., Abikar, K., Ing, A., Gordon, J.: Dysfunction of humoral immunity in anergic surgical patients. J. Clin. Immunol. (In press)
7. Christou, N.V., McLean, A.P.H., Meakins, J.L.: Host defense in blunt trauma: Interrelationships of kinetics of anergy and depressed neutrophil function, nutritional status, and sepsis. J. Trauma 20:833, 1980
8. Superina, R.A., Christou, N.V., Meakins, J.L.: Failure of neutrophil delivery into skin windows in anergic surgical patients. Surg. Forum 31:68, 1980
9. Miles, A.A., Miles, E.M., Buche, J.: The value and duration of defense reactions of the skin to the primary lodgement of bacteria. Br. J. Exp. Pathol. 38:79, 1957
10. Shizgal, H.M., Milne, C., Spanier, A.H.: The effect of nitrogen-sparing intravenously administered fluids on postoperative body composition. Surgery 85:496, 1979
11. Christou, N.V., Superina, R.A., Broadhead, M., Meakins, J.L.: Postoperative depression of host resistance: Determinants and effect of peripheral protein-sparing therapy. Surgery (In press)
12. Van Epps, D.E., Frierson, J.A., Williams, R.C.: Immunological studies of anergic patients. Infect. Immun. 10:1003, 1974
13. Bohnen, J.M.A., Christou, N.V., DeVoe, I., Chiasson, L., Meakins, J.L.: Anergy secondary to intraperitoneal sepsis: An animal model. Surg. Forum (In press)