In Vivo *and* In Vitro *Humoral Immunity in Surgical Patients*

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***In vivo* and *in vitro* humoral immunity was studied in surgical patients. Laboratory controls (LC), delayed type hypersensitivity (DTH) skin test reactive (HR), and anergic (HA) patients were immunized with tetanus toxoid. Maximum *in vivo* antibody levels occurred 14 days after immunization. Eighty-six, 47, and 17% of LC, HR, and HA subjects, respectively, showed a positive response ($\chi^2_2 = 21.1$ with Yates, $p < 0.0005$). Peak *in vitro* antibody production in unstimulated lymphocyte cultures occurred at day 6 after immunization. Antibody responses *in vitro* were reduced in all surgical patients, worst in HA, and correlated quantitatively with *in vivo* antibody responses at day 14 (Spearman rank correlation = 0.794, $p < 0.001$). Total IgG production *in vitro* was not decreased; 595, 1080, and 1538 ng IgG/culture were produced by LC, HR, and HA, respectively. These data demonstrate decreased *in vivo* and *in vitro* humoral immunity in all surgical patients, worst in those with decreased DTH responses. There is a kinetic and quantitative correlation between *in vivo* and *in vitro* responses, the latter being a biologic reflection of the integrity and magnitude of the *in vivo* process. Finally, failure to produce specific antibody is not due to failure of total IgG synthesis.**

GRAM-NEGATIVE SEPSIS continues to be a major cause of morbidity and mortality in surgical patients. Estimates of incidence in hospitalized populations range from less than one to 10%.[1-3]. The overall case fatality rate is approximately 30%,[1] although it is clear that both incidence of sepsis and risk of death are higher in patients with more severe underlying disease.[4] The important pathogens currently causing serious sepsis are normal commensal and nosocomially acquired gram-negative rods on epithelial surfaces, which cause illness by exploiting opportunities created by invasive medical procedures and defects in systemic host defence mechanisms. The identification of patients with high risk for sepsis has therefore been an important goal, so that properly stratified patients may be studied to identify defects in host resistance mechanisms. Knowledge of the nature and extent of such defects is a prerequisite to future therapeutic developments.

We have previously described several biological manifestations of acquired immunodeficiency in surgical patients.[5,6] Decreased delayed type hypersensitivity (DTH) skin testing with recall antigens has been correlated with increased risk of sepsis and mortality in 2202 surgical patients.[7] Because humoral immunity is important in host defence against gram-negative organisms, we examined the antibody responsiveness of surgical patients classified according to DTH skin testing. Using tetanus toxoid (TT) immunization as a test procedure, the *in vivo* antibody responsiveness of all surgical patients was found to be decreased, worst in anergic subjects.[8,9] To further describe the defect in humoral immunity, we have performed *in vitro* studies of total IgG and anti-TT antibody production in cultures of lymphocytes, unstimulated by antigen or polyclonal B-cell activators, from TT immunized surgical patients. These studies demonstrate abnormalities of both *in vivo* and *in vitro* antibody responsiveness in all surgical patients, worst in those with reduced DTH responses. Furthermore, a significant correlation is shown between *in vivo* and *in vitro* humoral immune responses.

Materials and Methods

Patient Population

Surgical patients on the wards and the surgical intensive care unit of the Royal Victoria Hospital were studied. DTH skin tests were performed by the intradermal injection of 0.1 ml of five recall skin test antigens. The antigens were Candida (1:100), mumps antigen (undiluted), purified protein derivative (5 TU), Trichophyton (100 PNU), and Varidase (100 U). A positive response was defined as induration equal to or greater than 5 mm at either 24 or 48 hours after injection. Patients responding to two or more antigens were classified as hospital reactive

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(HR) and those responding to no antigens as hospital anergic (HA). No subjects responding to a single antigen were studied. Healthy laboratory personnel were studied as controls (LC).

Sixty-six subjects were studied, including 21 LC (age 21–65 years, median 41), 15 HR (age 41–79 years, median 68) and 30 HA (age, 43–90 years, median 68). The patients represent a diagnostically heterogenous group of general surgical patients. Subjects with blunt, penetrating, or thermal trauma were excluded. No patients received corticosteroids, chemotherapy, or radiotherapy during the period of study, or for 1 year previously. Patients receiving blood products during the study, except packed red blood cells, were excluded. No subject had received a TT booster in the previous 2 years.

The study was approved by the Ethics Review Committee of the Royal Victoria Hospital. Informed consent was obtained.

Protocol

Patients were classified as HR or HA on the basis of skin testing at the time of immunization. All subjects received 0.5 ml of aluminum phosphate adsorbed TT intramuscularly (Lederle Laboratories, NY). The day of immunization was designated day 0. Serum samples were obtained the day of immunization and at intervals thereafter. Serum was stored at 4 C with 0.025% sodium azide until assay. Peripheral blood was obtained at intervals before and after immunization from 9 LC, 5 HR, and 4 HA subjects for *in vitro* studies.

Cell Preparation and Culture

Defibrinated blood was centrifuged and serum removed. The remaining blood was diluted with an equal volume of Hanks balanced salt solution with 0.01 M HEPES buffer (HBSS), layered on Ficoll-Paque (Pharmacia, Montreal, Quebec, specific gravity = 1.070), and centrifuged. Interface mononuclear cells (MNC) were harvested and washed three times with HBSS. After incubating in HBSS at 37 C for 1 hour to remove cytophilic immunoglobulin, cells were washed twice more in HBSS. Differential counting was performed in Turk's solution in a hemocytometer. Cell viability by trypan blue exclusion exceeded 95%. MNC were suspended at 2×10^6 lymphocytes/ml in the following medium: RPMI 1640 supplemented with 2% fetal bovine serum (FLOW laboratories, McLean, VA, lot 29101599), 0.01 M HEPES, 50 IU penicillin/ml, 50 mcg streptomycin/ml, (GIBCO, Grand Island, NY), 10 mM glutamine, 5 mcg/ml bovine insulin, 5 mcg/ml iron saturated human transferrin, and 5 ng/ml selenium (ITS®), Collaborative Research, Boston, MA). Triplicate cultures of 0.4×10^6 lymphocytes/well in a volume of 220 microliters were incubated in round-bottom polystyrene microtiter plates (Linbro, Flow Laboratories, McLean, VA) at 5% CO_2 in a humidified 37 C incubator. Cultures were not stimulated by either antigen or polyclonal B-cell activators. After 10 days, supernatants were harvested into polyvinyl chloride (PVC) microtiter plates and stored at −20 C until assayed.

Assay for Serum IgG Anti-tetanus Toxoid Antibody

A solid phase radioimmunoassay for IgG anti-TT antibody was adapted from Stevens and Saxon.[10] Affinity purified goat anti-human IgG heavy chains (Zymed Lab, Burlingame, CA) was radiolabelled with ^{125}I using a modification of Markwell and Fox.[11] Two hundred micrograms of antibody protein were reacted with one mCi of carrier free $Na^{125}I$ in a borosilicate glass tube previously coated with 10 micrograms of 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril. Unbound ^{125}I was removed by passage through a 10-ml Sephadex G25M column equilibrated with 0.1% bovine serum albumin (BSA) in phosphate buffered saline with 0.025% sodium azide (PBS-AZ). Specific activities of 1000 to 2000 cpm/ng protein were obtained with less than 5% unbound ^{125}I. Heavy chain specificity of the radiolabelled antibody was confirmed by incubating aliquots in PVC microtiter plates (Dynatech, Alexandria, VA) coated with chromatographically purified human IgG, IgM (Cappel Laboratories, Cochranville, PA), or BSA. Binding of radiolabelled antibody to IgG exceeded 40% of radiolabel added to each well. Binding to IgM and BSA was less than two and one per cent, respectively.

To assay serum samples for anti-TT, 25 microliter aliquots of soluble TT (Connaught Laboratories, Toronto, Ontario) at 10 mcg/ml in PBS-AZ were incubated overnight in the wells of round bottom PVC microtiter plates. After washing four times with PBS-AZ, 75 microliters of 1% BSA in PBS-AZ were incubated 2 hours. After washing four times, 25 microliter aliquots of serial log dilutions of serum samples in 0.1% BSA in PBS-AZ were placed in triplicate wells. After an 18-hr incubation followed by six washes, 120 ng of radiolabelled anti-human IgG in 30 microliters were placed in each well and incubated 18 hours. The wells were finally washed six times and counted individually in a Beckmann gamma-counter. All incubations were at room temperature and all washing with PBS-AZ. The quantities of solid phase TT and radiolabelled antibody were in excess for the serum dilutions used.

A standard curve was produced for each experiment using a serum known to contain 4 international units (IU) of IgG anti-TT/ml. Bound radioactivity in triplicate experimental wells was converted to IU anti-TT/ml serum *via* a linear regression formula of log-log transformed data from the standard serum. Serum and TT negative controls were included in each assay. Background binding

produced a level of detectability of 0.01 IU/ml. The arithmetic mean of triplicate assay wells at the experimental serum dilution nearest the midpoint of the linear regression line was taken as the antibody content. Serum samples from each donor were assayed at the same time. The *in vivo* serum antibody response was calculated as IU anti-TT/ml serum at day 14 after immunization minus IU anti-TT/ml at day 0.

Assay for Culture Supernatant IgG Anti-TT

Solid phase radioimmunoassay of culture supernatants for IgG anti-TT was performed similarly to assay of serum except that undiluted culture supernatants were incubated in the wells in place of diluted serum. Bound radioactivity was quantified as IU $\times 10^{-6}$ culture. The level of detectability was 0.01 IU $\times 10^{-6}$/culture. Geometric means of duplicate assay wells of triplicate culture supernatants are shown.

Assay for Culture Supernatant Total IgG

A solid phase competitive immunoradioassay was used to quantitate total IgG in culture supernatants. The wells of round bottom PVC microtiter plates were coated overnight with 50 microliters of chromatographically purified human IgG at 10 mcg/ml in PBS-AZ. After three washes with PBS-AZ, 75 microliters of 1% BSA in PBS-AZ were placed in the wells and incubated 2 hours. The wells were washed three times, and 25 microliters of undiluted culture supernatant placed in them. This was followed by 10 microliters of radiolabelled anti-human IgG at 2 mcg/ml in 0.1% BSA in PBS-AZ. After a 2-hour incubation at 37 C, the plates were washed six times with PBS-AZ and remaining bound radioactivity counted. Individual supernatants were assayed in duplicate. With each assay, a standard curve was prepared using known concentrations of human IgG in culture medium. Bound radioactivity *versus* the log of IgG concentration was linear over the range of standard IgG concentrations used. Bound counts per minute in experimental wells were converted to ng IgG/culture using a linear regression formula from the standard curve. Data from duplicate assay wells and triplicate culture supernatants were averaged arithmetically. Heavy chain specificity was demonstrated by the failure of chromatographically purified IgM or IgA to inhibit binding of the radiolabelled anti-IgG to the solid phase IgG. The same culture supernatants were assayed for anti-TT and total IgG.

Statistical Analysis

Least squares method of linear regression was used for the radioimmunoassay standard curves. Group data were averaged as geometric means. Comparisons between groups and correlations of *in vivo* and *in vitro* data were analyzed using chi square, Wilcoxon rank sum tests, and Spearman rank correlation.

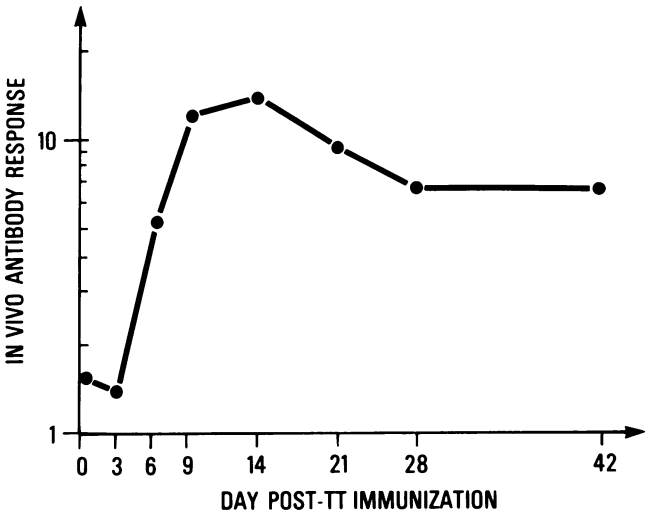

FIG. 1. Normal *in vivo* response to TT immunization. Four LC subjects were immunized on day 0 with tetanus toxoid. Geometric means of IU IgG anti-TT/ml serum at intervals after immunization are shown.

Results

Normal In Vivo *Anti-TT Response*

Four LC subjects were immunized with TT. The geometric mean levels of serum antibody at intervals from day 0 to 42 are shown in Figure 1. After a lag phase of 3 days, antibody content begins to rise, reaching a peak at day 14. Levels thereafter decline to a new plateau at day 28 to 42. For these individuals, the mean quantity of new IU/ml serum at day 14 was 17.8. Although the peak serum content is reached at day 14, the maximal synthesis rate *in vivo* occurs between days 3 to 9, indicated by the steepest slope of the response curve. During this period, there is an exponential increase in serum antibody levels.

Patient In Vivo *Anti-TT Response*

The *in vivo* antibody responses of surgical patients classified as HR or HA at the time of immunization are shown in Figure 2. Eighty-six per cent of LC produced more than 0.1 IU/ml, considered a normal response, compared to 47% and 17% for HR and HA subjects, respectively, (chi squared value with two degrees of freedom = 21.1 with Yates correction, $p < 0.0005$). The geometric mean antibody responses of LC, HR, and HA are 4.33, 0.12, and 0.03 IU/ml, respectively. There are statistically significant differences between LC *versus* HR, LC *versus* HA, and HR *versus* HA ($p < 0.05$, Wilcoxon rank sum tests).

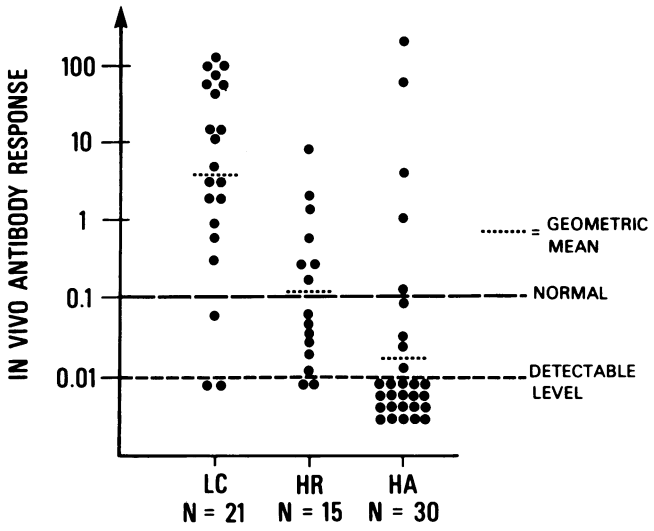

FIG. 2. *In vivo* response to TT immunization. Serum antibody response for LC, HR, and HA subjects was determined as IU IgG anti-TT/ml at day 14 after TT immunization minus day 0. The detectable level is 0.01 IU/ml, and a normal response ≥ 0.1 IU/ml.

Normal In Vitro *Anti-TT Response*

Production of specific IgG anti-TT antibody *in vitro* after *in vivo* immunization was studied in four LC subjects. After immunization, peripheral blood mononuclear cells were harvested from days 0 to 42 and cultured *in vitro* for 10 days. Culture supernatants were assayed quantitatively for cumulative antibody content. The results are shown in Figure 3. Prior to immunization no subject produced detectable quantities of antibody *in vitro*. Detectable antibody synthesis began 3 days after immunization and peaked at day 6. Lesser quantities of antibody were produced by MNC harvested 9 and 14 days after immunization. From day 21 onward, there was no detectable *in vitro* antibody production.

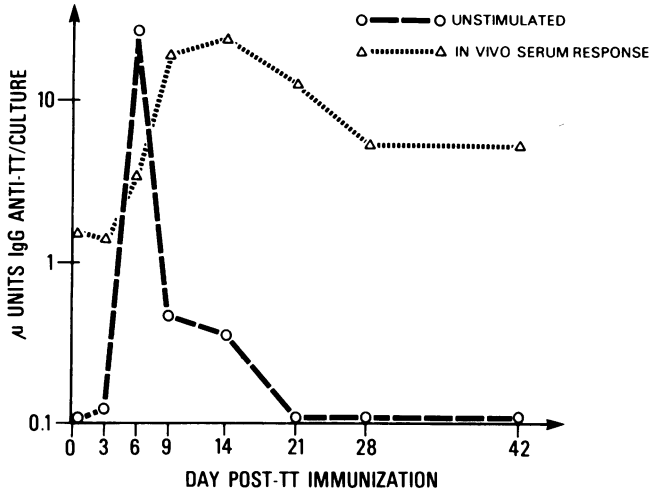

FIG. 3. Normal *in vivo* and *in vitro* response to TT immunization. Four LC subjects were immunized on day 0 with TT. Geometric means of *in vitro* (IU × 10^{-6}/culture) and *in vivo* (IU/ml serum) antibody responses at intervals after immunization are shown.

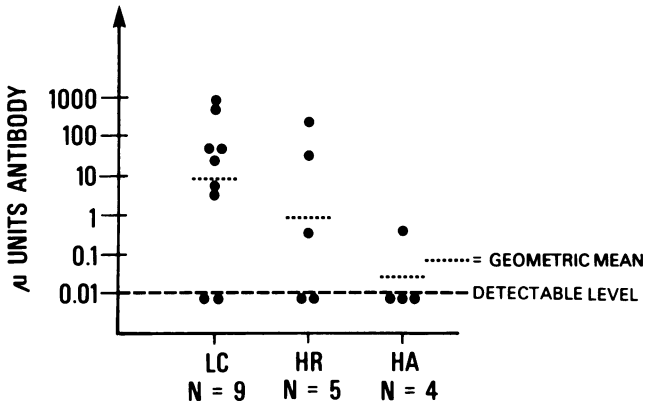

FIG. 4. *In vitro* response to TT immunization. Quantities of IgG anti-TT antibody (IU × 10^{-6}/culture) produced *in vitro* by MNC from LC, HR, and HA subjects 6 days after TT immunization are shown. The minimum detectable quantity is 0.01 IU × 10^{-6}/culture.

Patient In Vitro *Anti-TT Response*

Peripheral blood MNC were harvested from LC and surgical patients on days 0, 6, and 14 after TT immunization. No subject produced antibody *in vitro* before immunization. Quantities of antibody in culture supernatants from MNC harvested on day 6 are shown in Figure 4. Similar to the *in vivo* data, there is a graded response between LC, HR, and HA. Seven of nine, three of five, and one of four LC, HR, and HA subjects, respectively, produced detectable quantities *in vitro*. The geometric mean antibody production *in vitro* was 7.34, 0.78, and 0.02 IU × 10^{-6}/culture for LC, HR, and HA groups. The single anergic individual who produced antibody *in vitro* at day 6 was also the only anergic subject in this group to show a normal *in vivo* response, 1.1 IU/ml serum. Apart from this single occasion, lymphocytes from this subject and all other anergic subjects did not produce specific antibody *in vitro* at any time before or after TT immunization. At day 14, the mean *in vitro* antibody production was 0.26, 0.43, and <0.01 for LC, HR, and HA. These amounts are less than those produced at day 6 for all groups.

Data from a representative HA subject is shown in Table 1. This 75-year-old female was admitted for investigation of diarrhea. The final diagnosis was metastatic carcinoid syndrome. She was anergic to skin testing on admission and remained so throughout hospitalization. Laparotomy was performed on day 15 after TT immuinization. Peripheral blood MNC harvested at day 0, 6, 14, and 20 failed to produce detectable quantities of

TABLE 1. *HA Subject Response to TT Immunization*

Day Post-TT	Anti-TT Antibody		Total IgG
	In Vivo	*In Vitro*	
	IU/ml serum	IU × 10^{-6}/culture	ng/culture
0	0.01	<0.01	785
6	0.01	<0.01	831
14	0.04	<0.01	382
20	0.02	<0.01	2674

antibody *in vitro*. The *in vivo* antibody response was similarly modest, only 0.03 IU/ml.

Correlation Between In Vivo *and* In Vitro *Antibody Responses*

The relationship between *in vivo* and *in vitro* anti-TT antibody production was examined. A quantitative correlation was identified between peak antibody responses *in vivo* at day 14 and peak antibody production *in vitro* at day 6. Spearman rank correlation produced a significant correlation coefficient, $r_s = 0.794$, $p < 0.001$ (Fig. 5). Correlating antibody production *in vitro* at day 14 instead of day 6 with *in vivo* antibody responses yielded a much poorer relationship, $r_s = 0.392$, $p > 0.1$.

Total IgG Production In Vitro

To determine whether failure of specific IgG antibody production *in vitro* was due to failure of IgG synthesis, total IgG was assayed in the same culture supernatants in which anti-TT IgG was measured. The mean cumulative ng IgG/culture produced by MNC harvested 6 days after immunization of LC, HR, and HA subjects were 595, 1080, and 1538, respectively (Fig. 6). As shown, MNC from patient groups synthesizing little anti-TT IgG produced quantities of total IgG comparable to normal. Similar amounts of total IgG were also produced by MNC from all groups before and at 14 days after immunization. Table 1 shows the ng IgG/culture produced by MNC harvested at intervals after immunization from the representative HA subject previously discussed.

Discussion

These data show decreased *in vivo* humoral immunity to TT in all surgical patients, worst in those with reduced DTH responses. Abnormalities in several aspects of inflammation and immunity have previously been associated with decreased DTH responses: neutrophil chemotaxis and bactericidal function are decreased;[12,15] there are inhibitors of chemotaxis present in serum from anergic patients;[13] and cell delivery to *in vivo* cutaneous abrasions fitted with skin window chambers is reduced.[14] Examination of lymphocyte function showed reduced T-cell percentages in peripheral blood, and a serum inhibitor of sheep red blood cell rosette formation by T-cells.[15] Despite abnormal numbers of T-cells in patients with decreased DTH responses, *in vitro* T-lymphocyte function, measured by mixed lymphocyte culture, cell mediated lympholysis, and antigen stimulation, seems normal.[15,16] In the context of the surgical patient, we therefore regard

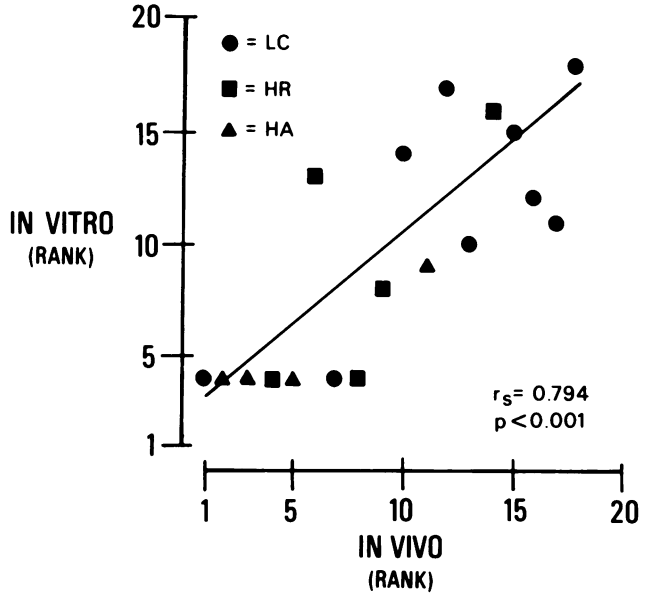

FIG. 5. Correlation of *in vivo* and *in vitro* antibody responses. *In vivo* IgG anti-TT response at day 14 and *in vitro* IgG anti-TT production at day 6 were correlated using Spearman rank correlation, ($r_s = 0.794$, $p < 0.001$). The data were plotted as ranks, and least squares method linear regression used to derive the graphic relationship between the ranks.

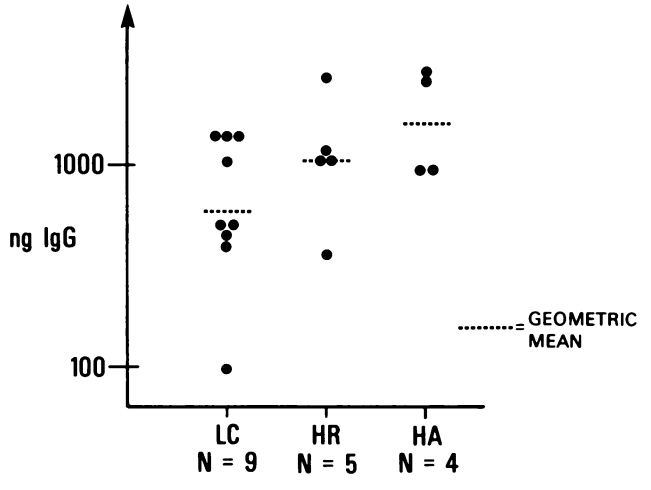

FIG. 6. *In vitro* total IgG production. Quantities of total IgG (ng/culture) produced *in vitro* by unstimulated MNC from LC, HR, and HA subjects 6 days after TT immunization are shown.

DTH responses not as a specific test of cell mediated immunity, but rather as a manifestation of a broad based acquired immunodeficiency syndrome, predisposing to bacterial sepsis.

There is a continuum of host defense abnormalities that parallels severity of illness in surgical patients. The inverse correlation between skin test results and sepsis and mortality[7] shows that DTH testing can mark the position of patients on this continuum. Other tests of immune function can then be related to DTH skin test responses and severity of illness. This study has thus shown that the continuum of decreased humoral immunity in surgical patients parallels depressed DTH skin testing.

The normal *in vitro* response to TT immunization has previously been studied. As soon as 5 days after immunization, a population of B-cells appears in the circulation that spontaneously secretes anti-TT *in vitro*.[17] Help from polyclonal B-cell stimulants or T-cells is not required. This population rapidly declines and is undectable after 2 weeks.[18] Our data confirms the transient appearance of spontaneous antibody secreting cells in the blood after TT immunization. In surgical patients who did not show a serum response, such cells could not be detected.

The following observations suggested that the *in vitro* production of antibody reflected the process of an *in vivo* antibody response. First, a comparison of the *in vivo* antibody responses measured at day 14 and the peak *in vitro* antibody production at day 6 showed similar graded responses between the study groups. Second, the period of peak *in vitro* antibody production coincided temporally with the period of maximal antibody synthesis *in vivo*. Finally, the only HA patient responsive *in vivo* was also the only in the HA group to produce antibody *in vitro*. A direct correlation was thus demonstrated between quantities of antibody produced *in vivo* by day 14 and *in vitro* by unstimulated blood MNC at day 6 after immunization. Although *in vitro* humoral immune function is usually studied by stimulating MNC with antigen and/or polyclonal B-cell activators, our observations[19] indicate that quantitative correlation of antibody production *in vivo* is better with unstimulated than with stimulated MNC cultures. We conclude from these observations that the population of spontaneous antibody secreting B-cells present in the circulation between 3 and 14 days after immunization with TT are a direct reflection of the *in vivo* humoral immune process. Quantitative studies of antibody synthesis by such cells can be used as a measure of *in vivo* humoral immune responses.

A correlation between *in vivo* antibody responses and production of antibody *in vitro* by blood MNC does not imply that circulating lymphocytes are the major site of *in vivo* antibody synthesis. Assuming MNC produce similar quantities of antibody *in vitro* and *in vivo*, it can be calculated that the lymphocytes present in blood at the time of peak *in vitro* antibody synthesis could account for less than 10 per cent of the total serum response. Therefore, the bulk of antibody production *in vivo* probably occurs in sites other than the blood, for example, lymph nodes, spleen, or bone marrow.[20] The appearance of spontaneous antibody producing cells in the blood may represent lymphocyte traffic between these anatomic sites. Regardless of the major site of antibody synthesis *in vivo*, the present study suggests that blood MNC can be used to study the dynamics and integrity of humoral immune responses in surgical patients.

The production of similar quantities of total IgG by MNC from both antibody responsive and unresponsive subjects shows that failure of antibody production is not due to failure of IgG synthesis. Because recent *in vivo* antigenic priming is required for spontaneous *in vitro* antibody synthesis,[17] failure of *in vivo* antigen recognition and/or lymphocyte activation may explain the absence of antibody production in unresponsive surgical patients. Previous studies have indicated a block in lymphocyte activation as one explanation for immunodeficiency associated with depressed DTH responses.[16] Whether one or several basic mechanisms produce the diverse manifestations of acquired immunodeficiency in surgical patients is not yet known.

The underlying etiology of decreased antibody responses in the heterogenous patient group reported here is unknown. Several conditions of surgical interest can alter humoral immunity. Most reports discuss only nonspecific aspects of the humoral immune system, such as serum immunoglobulin levels and B-cell numbers.[21] Data on alterations in specific humoral immunity due to illness have recently been reviewed[21] but are inconclusive. Malignancy, old age, and surgery may or may not affect various aspects of humoral immunity. Malnutrition, frequently present in surgical patients,[22] affects antibody responses to some antigens and not others.[21] Unfortunately, although there is a relationship between reduced DTH responses and malnutrition, skin testing does not accurately assess an individual's nutritional status.[23] Although not definitely demonstrated, it can generally be concluded that several underlying disease processes contribute to the immunodepression in any given surgical patient.

If the failure of humoral immunity to TT observed in this study implies a failure of antibacterial antibody responses in surgical patients, passive immunization with preformed antibodies, which has long been a goal of applied immunomodulatory therapy, is indicated. A major difficulty is the source of antibodies.[24] Human antisera against *Pseudomonas*[25] and *Escherichia coli*[26] have proven effective in human trials. The development of hybridoma technology by Kohler and Milstein in 1975[27] has offered

a new source of agents for passive immunization, monoclonal antibodies. Using murine monoclonal antibacterial antibodies, promise in prophylaxis against sepsis has been shown in animal models.[28] The blood lymphocytes spontaneously secreting antibody may be a useful source of cells for the production of human monoclonal antibodies, provided the kinetics of *in vitro* antibody production are understood.

In conclusion, we have demonstrated decreased *in vivo* and *in vitro* humoral immunity in a heterogenous group of surgical patients. There is a kinetic and quantitative correlation between *in vivo* and *in vitro* responses, the latter being a biologic reflection of the magnitude and integrity of the *in vivo* process. Failure to produce specific antibody is not due to failure of total IgG synthesis. Although there will be important new applications for active immunization against bacteria, further knowledge of humoral immune processes in man is required to facilitate exploitation and modulation of *in vivo* responses. The development of human monoclonal antibacterial antibodies for passive immunization will be facilitated by increased understanding of *in vitro* humoral immune responses.

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Discussion

DR. LOREN J. HUMPHREY (Shawnee Mission, Kansas): Dr. Nohr and colleagues have stratified surgical patients on the basis of response to skin test antigens and found that specific production of antitetanus toxoid antibody *in vitro* and *in vivo* correlated with the skin testing. Thus, humoral immunity to a specific antigen was depressed at a time when the general immunoglobulin level was normal.

In 1975, Slade, Simmons, and co-workers measured 12 parameters of immune function and found that all *in vitro* functions studied, such as total peripheral blood lymphocyte count, B-cell count, T-cell count, and lymphocyte blastogenesis to mitogens, fell with induction of anesthesia and continued to fall during and after surgery. Thus, cell immunity is depressed as well.

In 1970, Dr. Frederickson and I showed that anesthesia and high levels of oxygen *in vitro* caused a decrease in production of antitetanus toxoid antibody. Interestingly, these could be rejuvenated appropriately *in vitro* to produce antitetanus toxoid antibodies again.

Of further interest to surgeons is the fact that it has been shown that tumor burden as well decreases immune responsiveness.

Why do these various agents cause immunosuppression, and what can we do to prevent this? Certainly, as shown by Dr. Nohr and his