The humoral immune response after thermal injury: An experimental model

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Background. *Severe thermal injury is associated with major alterations in cell-mediated immunity. Because most B-cell responses are regulated or critically dependent on T-cell help, it is not surprising that many studies have also shown a variety of defects in humoral immunity after thermal injury. However, the nature of the relationship between the in vitro ability to produce antibody and subsequent in vivo responses remains unclear.*
Methods. *With a murine model of thermal injury, the primary and secondary humoral immune response to tetanus toxoid (TT) was examined during a 6-week period after sham burn or burn injury. Serum anti-TT titers and the numbers of anti-TT-secreting splenocytes were determined.*
Results. *Splenocytes from burned animals displayed normal or decreased TT-specific immunoglobulin (Ig) M plaque formation. In contrast, however, IgG plaque formation was persistently increased for up to 6 weeks after thermal injury, suggesting a switch from IgM to IgG antibody production. Conversely serum titers of TT-specific IgG antibody were persistently lower in burn, compared with sham groups. Changes in serum immunoglobulin levels did not account for this marked discrepancy between enhanced in vitro IgG plaque formation but impaired in vivo levels of TT antibody.*
Conclusions. *The data suggest that thermal injury is associated with a diminished ability to propagate and maintain a normal IgG antibody response, despite the presence of normal or increased numbers of antigen-specific B cells. (SURGERY 1994;115:341-8.)*

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DESPITE MAJOR ADVANCES IN the care of thermally injured patients, sepsis remains the most important cause of death.[1, 2] The prevalence and severity of infective episodes in these patients are largely secondary to the serious alterations in host defense mechanisms that follow thermal injury. A broad spectrum of defects in immune function has been documented after thermal injury.[3-6] In particular, major impairment of cell-mediated immunity is felt to play an important role in this increased susceptibility to infection.[7-9]

Because most B-cell responses are regulated or critically dependent on T-cell help, it is not surprising that many studies have also shown a variety of defects in humoral immunity after thermal injury.[8, 10, 11] The nature of the relationship between the in vitro ability to produce antibody and subsequent in vivo responses remains unclear. The importance of the humoral immune response in preventing infection after thermal injury, however, is highlighted in the work of Baker,[12] who demonstrated a close relationship between impaired humoral immunity and the subsequent development of severe sepsis.

The aim of this study was to examine the ability of thermally injured animals to initiate, propagate, and maintain a humoral immune response to a T-cell-dependent bacterial antigen after both primary and secondary immunization. In particular, we examined the long-term relationship between total serum immunoglobulin (Ig) levels, the in vivo response to tetanus toxoid (TT), reflected by the serum concentrations of TT-specific antibody, and the in vitro response to the same antigen by quantitation of TT-specific B cells.

Supported by NIH grant RO1GM3563308, the Ainsworth Scholarship, University College, Cork, Ireland, and the Brigham Surgical Group Foundation.

Presented in part to the American College of Surgeons Surgical Forum, Chicago, Ill., October 20-25, 1991. The proceedings of the meeting, including an abstract of this paper entitled, "In vivo and in vitro humoral immunity following thermal injury," were published in the Surgical Forum 1991;42:74-6.

Accepted for publication Dec. 21, 1992.

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0039-6060/94/$3.00 + 0 11/56/49226

METHODS

Immunization. Male A/J mice, 4 and 7 weeks of age, were purchased from Jackson Laboratories, Bar Harbor, Maine. Animals were acclimatized for 1 week under controlled conditions with mouse food and water ad libitum. The younger mice (now 5 weeks of age) received either a primary immunization of TT (1 Loeffler units [Lf U]; Wyeth Laboratories, Inc., Philadelphia, Pa.) diluted in 0.5 ml of saline solution, administered by intraperitoneal injection, or a control injection of vehicle only. This group received a similar secondary immunization at 8 weeks of age immediately before randomization and thermal injury as outlined later. The remaining animals received a single primary immunization of TT or vehicle at the time of randomization and burning (that is, 8 weeks of age).

Burn model. At 8 weeks of age, all animals were randomized into burn and sham groups and anesthetized with pentobarbital (1.25 mg mouse^{-1} in 0.75 ml saline solution) by intraperitoneal injection. Animals were shaved over their backs and placed in a specially constructed mold that exposed 25% of the total body surface area. The mold, containing an anesthetized animal, was then lowered for a period of 9 seconds into water at either 22° C for sham burn or 90° C for burn groups. The latter injury resulted in a histologically proven full-thickness burn. All animals were then dried and resuscitated with 1 ml of saline solution (intraperitoneally). Thereafter, animals were killed in groups of five at 0, 1, 2, 3, 4, and 6 weeks after injury. Blood was sampled by open cardiac puncture, and spleens were removed for assay as outlined later.

Care of animals and all animal procedures were carried out in accordance with National Institutes of Health guidelines and with review and approval of the Standing Committee on Animals, Harvard Medical Area.

Cell preparation. Individual splenocyte suspensions were obtained by gently teasing the freshly harvested spleens apart with sterile forceps and removing the coarse debris. Splenocytes were suspended in RPMI-1640 medium with 2 mmol/L l-glutamine, 1% antibiotic/antimycotic (penicillin 10,000 units, streptomycin 10,000 μg, amphotericin 2.5 μg ml^{-1}) and 10 mmol/L HEPES buffer (all reagents for washing and culture were purchased from Grand Island Biological Co., Grand Island, N.Y.). Cell suspensions were centifuged three times in this medium at 1500 rpm for 10 minutes. After the final wash, cells were suspended in the same medium containing 5% heat-inactivated fetal bovine serum (56° C, 30 min.). Mononuclear cells were counted with Turk's solution, and viability was assessed with the trypan blue exclusion test. Cell viability was consistently more than 95%. Cells were finally suspended at a dilution that was found to yield the maximum clarity in the subsequent enzyme-linked immunosorbent spot assay (ELISA spot assay). This was different for the IgG and IgM assays as outlined later.

ELISA spot assay. Tissue culture plates (24 round 1.5 cm diameter wells/plate; Nunc, Inc., Naperville, Ill.) were pretreated with 1 ml of 0.2% (vol/vol) glutaraldehyde (Sigma Chemical Co., St. Louis, Mo.) in 0.1 mol/L phosphate-buffered saline solution (pH 7.4) (PBS), before being coated with antigen. TT antigen prepared by the State Laboratories of the Massachusetts Department of Public Health, Boston, Mass. (0.4 ml, 20 Lf U ml^{-1}, diluted in 0.9% saline solution) was added to the wells and incubated overnight at 4° C. Some wells were coated with the diluent only and acted as a negative control. Excess antigen was washed from wells with three washes of 0.05% polysorbate 20 (Tween 20; Sigma Chemical Co.) in PBS (PBS-Tween). Plates were then blocked with 1% goat serum and bovine serum albumin in PBS for 1 hour at 37° C. After being blocked, wells were washed three times with PBS before the addition of the splenocytes. It was found that optimal clarity of subsequent ELISA spot formation was obtained by adjusting the concentration of the cell suspensions according to immunization group (primary or secondary), IgG or IgM assay, and duration after immunization. Cell suspensions were therefore plated at concentrations ranging from 1×10^4 to 1×10^7 ml^{-1}.

The remainder of the ELISA spot assay was carried out with the previously described technique of Sedgwick and Holt.[13] Cell suspensions were incubated at 37° C in a 5% Co_2-enriched atmosphere for 1.5 hours. After incubation, the plates were tapped empty and immediately washed three times with PBS-Tween. Wells were further incubated for 1 hour at 37° C after the addition of 0.4 ml of alkaline phosphatase-conjugated antimurine IgG or IgM antibody (30 mg/ml, diluted 1/1000 and 0.1% bovine serum albumin in PBS; Boehringer Mannheim GMBH, Mannheim, Germany). After incubation with the enzyme-conjugated antibody, plates were washed free of unbound antibody with PBS-Tween. An insoluble chromogenic solid-phase substrate was then added. The substrate used was 5-bromo-4-chloro-3indolyl phosphate (5-BCIP; Sigma Chemical Co.) in a 0.5% low-melt agarose solution. The substrate/agarose mix (0.4 ml) was added to each well and allowed to gel. A spot of brilliant blue color developed at each site of putative antibody formation, where a TT antibody-producing cell had settled. Plaques were easily seen with the naked eye and approximately 2 mm in size. For each mouse and each antibody type (IgG or IgM), four wells were assayed. Three were coated with TT antigen, and the fourth was coated with vehicle only. ELISA spot plaques were enumerated for the three TT wells, and the average was taken. Wells coated with diluent only consistently showed no IgG plaque formation

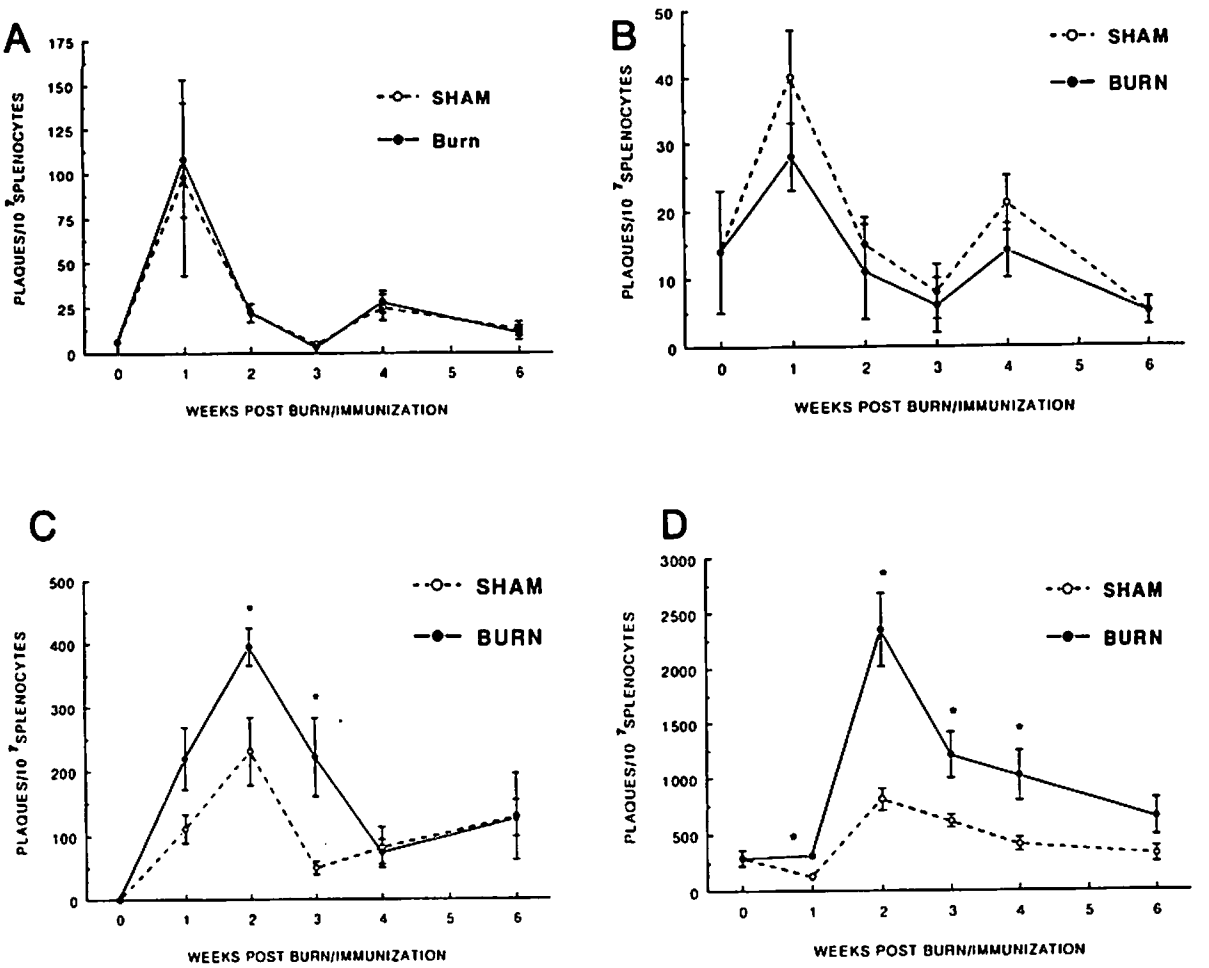

Fig. 1. TT-specific plaque formation sham and burn groups, measured by ELISA spot assay: **A,** IgM after primary immunization; **B,** IgM after secondary immunization; **C,** IgG after primary immunization; and **D,** IgG after secondary immunization. Values in all figures expressed as mean ± SEM. $*p < 0.05$ compared with sham control.

and fewer than 10% of TT-coated wells for the IgM assay.

Tetanus toxoid ELISA assay. Serum samples were assayed for IgG and IgM TT-specific antibody with a modification of previously described techniques.[14] Briefly, 96-well ELISA plates (Costar Corp., Cambridge, Mass.) were initially coated with antigen and blocked as for the ELISA spot assay. Samples were diluted 1:100 in PBS-Tween and plated in triplicate (100 μl of substrate solution [p-nitrophenyl phosphate]; Sigma Chemical Co.). The enzyme reaction yielded a bright yellow color change, which was read at an optical density of 405 nm on a microplate reader (UVMAX; Molecular Devices Corp., Menlo Park, Calif.). The concentration of TT antibody in each serum sample was measured in triplicate, by comparison with the outer diameter of a standard curve set up on each plate. Hyperimmune serum was used for standard curves and given an arbitrary value of 100 units/ml of TT antibody. Calculation of the concentration of TT antibody for each sample was performed with the SOFTmax computer program (Molecular Devices Corp.).

Immunoglobulin concentration. Serum IgG and IgM levels were measured by radial immunodiffusion (RID) with previously described techniques[15] with commercially available reagents (all antibodies and reagents used in the RID assays were purchased from The Binding Site, Inc., San Diego, Calif.). Briefly, RID plates were coated with a 1% agarose gel (40° C melting point) containing 3% polyethylene glycol 6000. Antimurine IgG or IgM (70 mg/ml) was added to give a final antibody concentration of 2 μl/cm^2 of gel surface area for the IgG assay and 2.4 μl/cm^2 for IgM estimation. Five-microliter wells were made in the agarose/antibody gel with a punch. The diluted samples and a standard curve with use of a calibrator solution with a known concentration of IgG or IgM were then added to the wells. On completion of RID, the diameter of the

ring of precipitation was measured with a magnifying eyepiece containing a calibrated graticule. Samples were measured in duplicate, and the mean of the two estimations was obtained. A standard curve was constructed by plotting the square of the diameter of the precipitation rings against the known concentration of immunoglobulin obtained from the calibrator solution. The square of the diameter of the samples was then plotted against the line constructed from the standards and the concentration of the samples obtained.

Statistics. Statistical analysis was performed with the Stata statistics program (Computer Resource Center, Santa Monica Calif.). The Mann-Whitney two-sample statistic was used to compare groups, and significance was taken at the 95% confidence level ($p = < 0.05$).

RESULTS

In vitro response. The in vitro response to TT as measured by TT-specific plaques is shown in Fig. 1, *a* through *d*. TT-specific IgM plaque formation was similar in sham and burn groups after primary immunization (Fig. 1, *a*). Although there was also no significant difference after secondary immunization, a definite trend was noted toward increased plaque formation in the sham-burn group (Fig. 1, *b*).

In complete contrast, however, a marked increase occurred in both primary and secondary IgG plaque formation after thermal injury. Fig. 1, *c* and *d*, represent TT-specific IgG plaques after primary and secondary immunization, where thermal injury is associated with a threefold increase in plaque formation 2 weeks after burning. This trend was maintained throughout the study period, with burn groups consistently showing enhanced plaque formation compared with the sham-burned controls.

In vivo response. The in vivo response, however, as measured by serum levels of TT-specific IgM antibody was markedly deficient in burn groups (Fig. 2, *a* and *b*). This was evident after primary immunization (Fig. 2, *a*) where thermal injury was associated with a reduction in TT-specific antibody throughout the first 4 weeks after injury. In addition, burned animals showed complete failure of their secondary IgM response compared with sham-burned controls (Fig. 2, *b*). Serum IgG TT antibody was also reduced after both primary and secondary immunization in the burn groups (Fig. 2, *c* and *d*, respectively). It is interesting to note that the difference, rather than decreasing with time, in fact became more marked, especially after secondary immunization (Fig. 2, *c* and *d*). The secondary response to TT in burn groups showed complete failure of propagation after the initial peak at 1 week after injury. Sham-burned animals, however, displayed normal propagation of their response, maintaining high antibody titers through week 6 after immunization.

Total immunoglobulin. Total serum levels for IgG and IgM were reduced in the burn groups after primary and secondary immunization. Serum IgM was maximally depressed between weeks 2 and 3 after thermal injury (Fig. 3, *a* and *b*). Thereafter, levels recovered so that IgM levels were similar for sham and burn groups from week 4. A similar pattern was seen in serum IgG levels, where the burn groups also displayed a transient fall compared with the sham-burned controls (Fig. 3, *c* and *d*). Burn injury was associated with, at most, a 30% reduction in total IgG during the first 3 weeks after injury. As with IgM levels, there was a recovery to control values by week 4.

DISCUSSION

During the last 10 years, major advances in burn care, fluid therapy, and nutrition have dramatically improved survival after major thermal injury. As a result, sepsis is now the most important cause of death, accounting for 50% to 70% of all deaths.[1, 2] Major thermal injury is associated with significant suppression of the immune response, resulting in that increased susceptibility to infection.[3-9] Although defects in cell-mediated immunity are a characteristic feature of thermal injury, its effects on the humoral immune response are less well defined.

Our results suggest that primary and secondary in vitro IgG responses to TT are persistently enhanced after thermal injury. Corresponding IgM responses did not, however, differ significantly from sham-burned controls. Simultaneous measurement of antigen-specific serum antibody titers showed deficient in vivo IgG responses, especially after secondary immunization. These data suggest that mechanisms for producing antigen-specific antibody after thermal injury are intact, although this does not appear to be translated into increased titers of antigen-specific IgG. Increased catabolism of immunoglobulin does not fully account for this dissociation between in vivo and in vitro responses, suggesting that other factors may be responsible for reduced serum levels of specific antibody after thermal injury.

Although many studies have shown a marked defect in humoral immunity after surgical[16] or mechanical trauma[17, 18] and thermal injury,[8, 10] the heterogenous nature of these injuries, combined with different study group characteristics, makes interpretation of data difficult. Further difficulties arise when one attempts to correlate results from assays of in vitro function with in vivo parameters of the humoral immune response. Although several reports suggest that thermal injury is associated with polyclonal B-cell activation,[8, 19] its effects on antigen-specific plaque formation are less clearly understood. In addition, it is unclear whether in vitro parameters of immune function accurately reflect the in

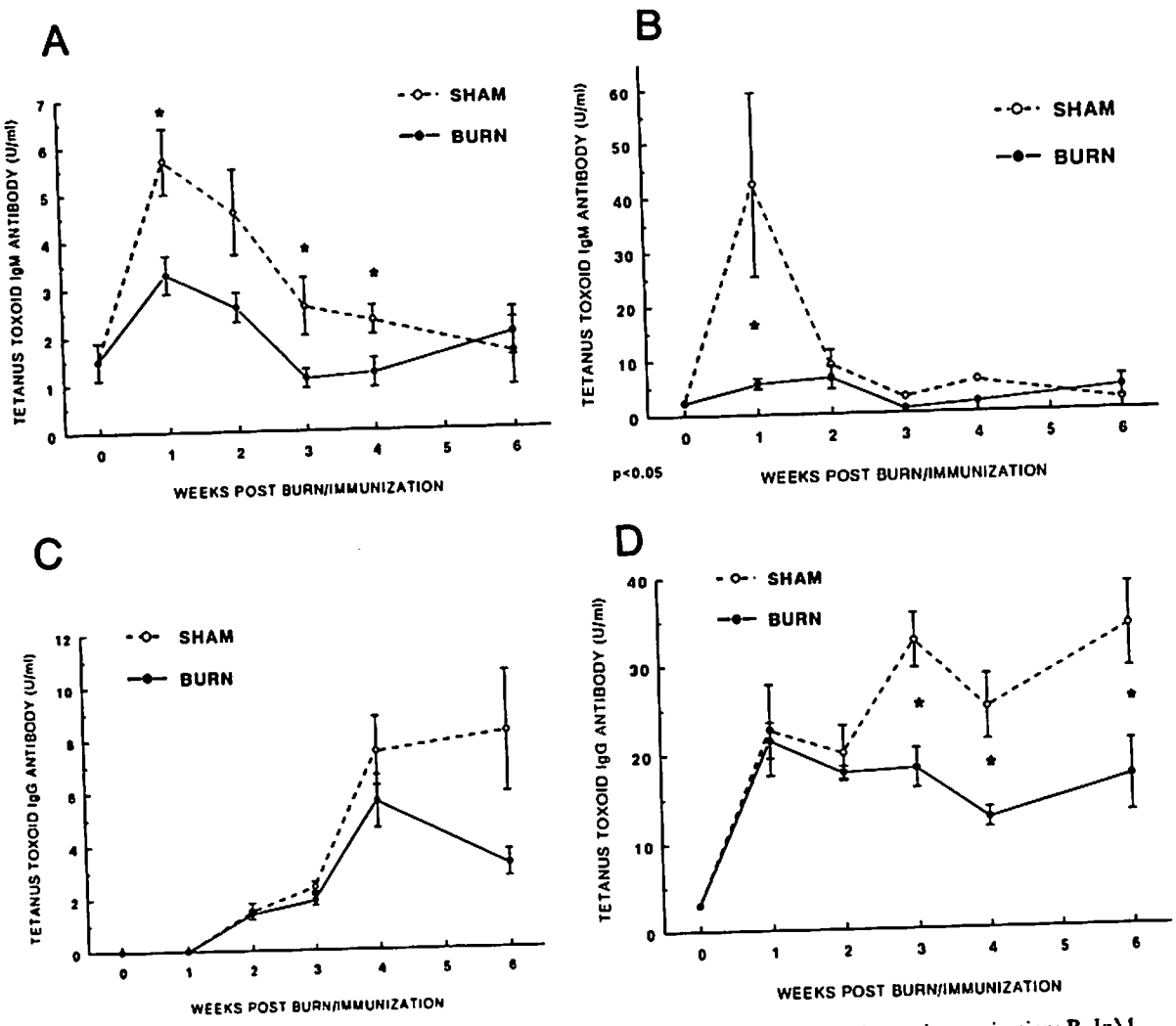

Fig. 2. Serum TT-specific antibody titers, sham and burn groups: **A**, IgM after primary immunization; **B**, IgM after secondary immunization; **C**, IgG after primary immunization; and **D**, IgG after secondary immunization.

vivo response as measured by serum levels of antigen-specific antibody. Investigators have used experimental models to address some of these questions. Many of these studies are notable for their use of short-lived responses to the antigens used[11, 20] (for example, sheep red blood cells), which do not accurately reflect the true nature of most clinically important antigens that induce a sustained antibody response. In this study, we simultaneously examined the effect of thermal injury on the primary and secondary in vitro and in vivo humoral immune response to a long-lived, T-cell–dependent antigen.

Although the in vitro IgM response to TT was similar in burn and sham groups after primary immunization (Fig. 1, *a*), a definite trend was noted toward reduced plaque formation after secondary immunization in the burn groups (Fig. 1, *b*). Thermal injury has previously been reported to be associated with a persistent reduction in nonspecific pokeweed mitogen, and interleukin (IL)-2–driven IgM secretion,[21] although the precise mechanisms are unclear. The kinetics of the response were similar in both groups, however, where the maximal response occurred 1 week after immunization and was followed by a rapid decline in plaque formation as B cells switched to IgG synthesis.

In contrast, in the present study, in vitro IgG responses were markedly enhanced in the burn groups after both primary and secondary immunization (Fig. 1, *c* and *d*). Once again, differences were most marked after secondary immunization where the burn groups showed a threefold increase in plaque formation 3 weeks after injury (Fig. 1, *d*). This difference was maintained throughout the study period where the burned animals consistently displayed enhanced plaque formation compared with the sham-burned controls. It therefore appears that burn injury was associated with a switch

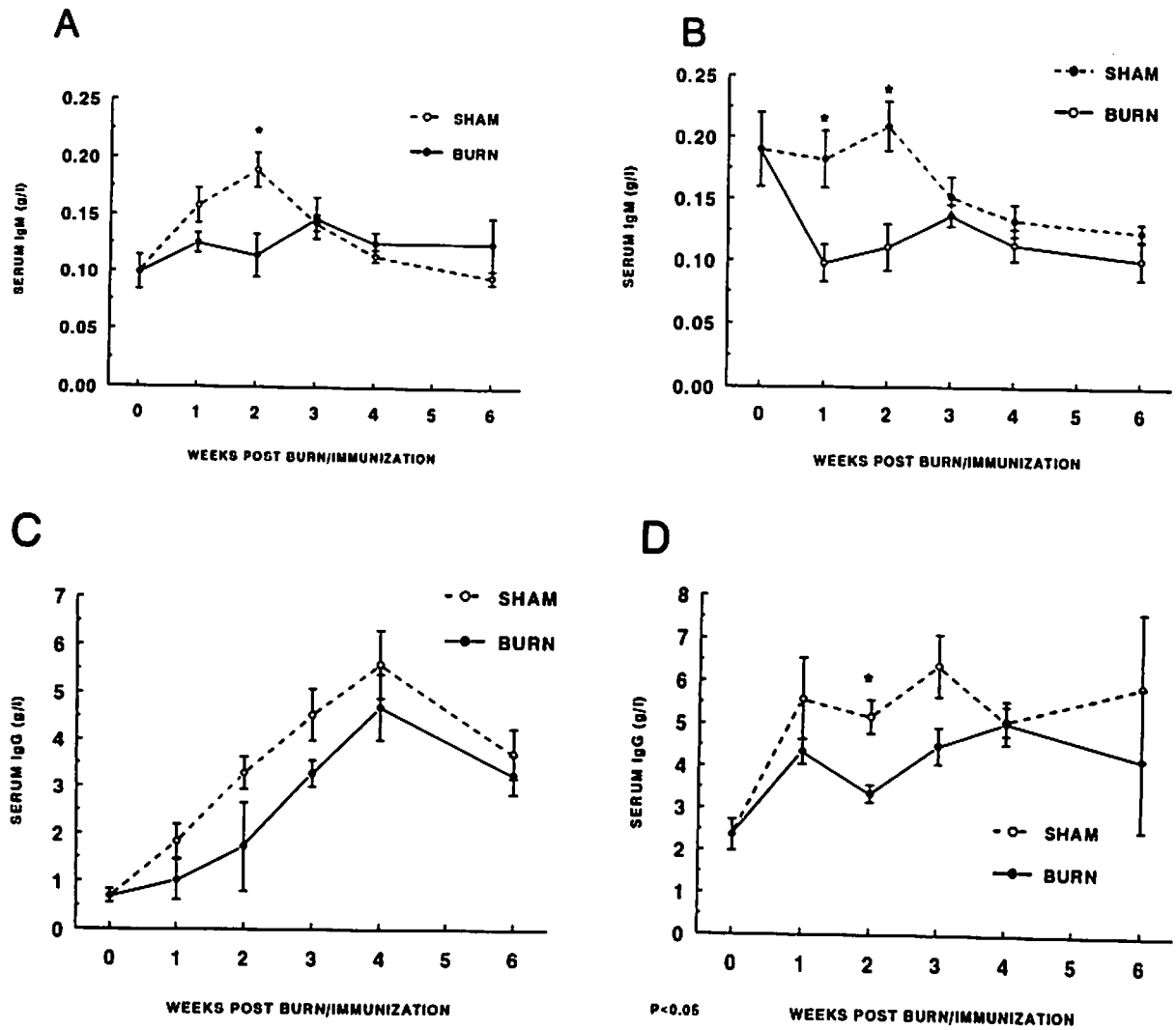

Fig. 3. Serum immunoglobulin levels measured by RID after immunization with TT: A, IgM after primary immunization; B, IgM after secondary immunization; C, IgG after primary immunization; and D, IgG after secondary immunization.

from IgM to IgG synthesis. Other investigators have similarly reported prolonged suppression of IgM in relation to IgG secretion in pokeweed mitogen-activated cultures from burn patients.[19, 22] This phenomenon has not, however, previously been reported for antigen-specific assays of in vitro function after thermal injury. Although it is unclear from these data why such a shift should occur, recent reports suggest that prostaglandin E_2 (PGE_2) may play a pivotal role. PGE_2 a macrophage-derived prostaglandin produced in response to a variety of stimuli, has recently been shown to trigger the switch from IgM to IgG synthesis in in vitro assays of immunoglobulin production.[23, 24] Mouse splenocytes stimulated with lipopolysaccharide and PGE_2 synergize with IL-4 to stimulate up to a 26-fold increase in IgE and IgG1 while simultaneously reducing IgM production. Using an ELISA spot assay technique, Ohmori et al.[25] have found that PGE_2 increased IgG1 and IgE production by increasing the number of cells producing antibody, rather than increasing the rate of antibody synthesis. It is interesting to note that thermal injury has long been shown to be associated with a marked increase in PGE_2 synthesis[26-28] and may therefore be responsible for this shift from IgM synthesis seen in the burn groups.

In this study, the in vivo response as measured by serum levels of IgM and IgG TT-specific antibody was deficient after thermal injury (Fig. 2, *a* through *d*). Differences between sham and burn groups were most marked after secondary immunization (Fig. 2, *b* and *d*). The secondary IgG response in particular exhibited failure of propagation beyond week 1 after burn injury (Fig. 2, *d*). These findings are consistent with previous studies that have reported a failure to propagate and maintain a normal in vivo IgG response after thermal injury.[10, 16] The failure to propagate and maintain a normal IgG response after thermal injury has been shown to correlate well with inadequate IL-2 produc-

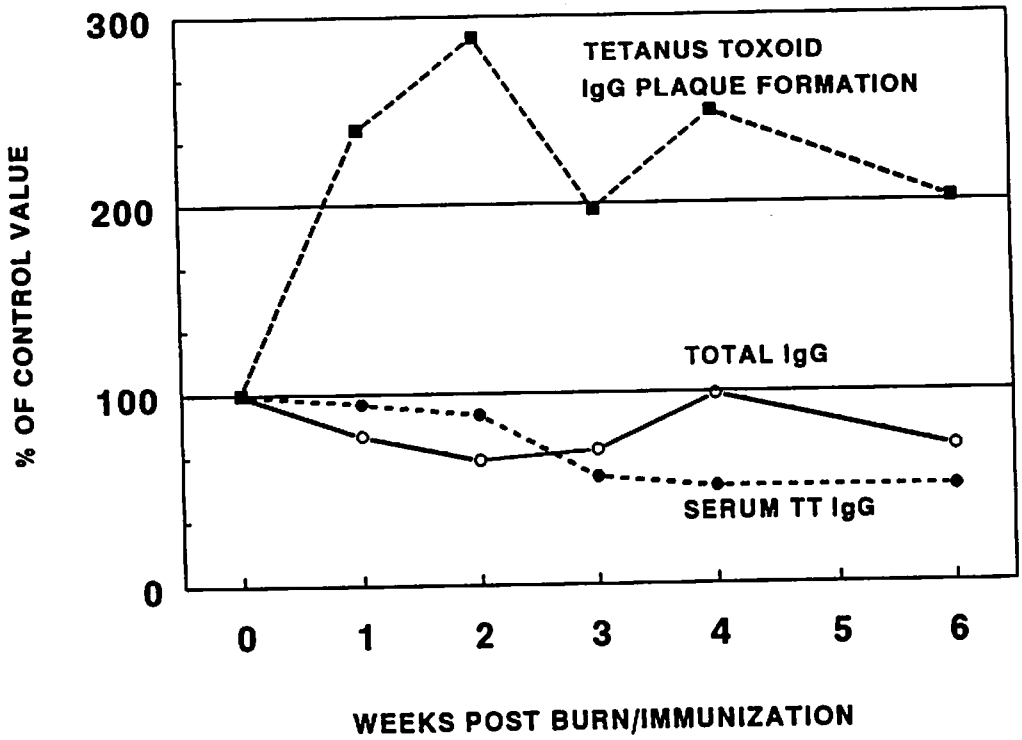

Fig. 4. Secondary IgG responses after thermal injury. Values are expressed as a percent of sham-burned controls.

tion in burn patients[10] and once again highlights the importance of an intact cellular immune response as a prerequisite for normal antibody production.

A further possible mechanism underlying this persistent reduction in serum levels of antigen-specific antibody might be increased protein catabolism, which is known to follow thermal injury, and has been shown to be responsible for reduced total immunoglobulin levels in burn patients.[11, 29] Increased protein catabolism secondary to protein exudation from the burn wound and increased intravascular degradation are felt to be responsible. We therefore also measured total immunoglobulin levels in burn and sham groups in an attempt to explain why thermal injury was associated with an enhanced in vitro IgG response but markedly deficient serum levels of the corresponding antibody. Burned animals showed a transient fall in both IgG and IgM after injury (Fig. 3, *a* and *b*). In this model, however, thermal injury was associated with, at most, a 30% reduction in immunoglobulin levels during weeks 2 and 3 after injury. Thereafter there was a recovery in both total IgG and IgM such that there was no significant difference by week 4. These data are consistent with previously published reports that have shown a similar transient reduction in total immunoglobulin levels after thermal injury in both human and experimental models.[11, 19, 29]

The small transient reduction in serum immunoglobulin seen here is unlikely to account, however, for the marked discrepancy between the in vitro and in vivo IgG responses after thermal injury. The secondary IgG humoral response after thermal injury is summarized in Fig. 4, where the data are represented as a percentage of the sham-burned controls. It is apparent that both total IgG and TT-specific antibody follow a similar pattern during the first weeks, that is, both are seen to fall, suggesting that increased protein catabolism is responsible at least in part for the reduction of specific antibody. However, although total IgG was seen to recover to control values by week 4, no such recovery was seen in TT-specific antibody titers. In addition, throughout this period TT-specific plaque formation was increased 200% to 300%. It therefore appears that, although burn injury was associated with much increased numbers of antigen-specific B cells, this did not translate into increased production of IgG antibody.

Although it is unclear from these data why there appears to be a long-lived dissociation between the in vitro and in vivo IgG humoral response after thermal injury, it suggests that either a reduction in the absolute quantity of antibody produced per cell or a failure of in vivo activation of B cells may be responsible. Because both the ELISA spot plaque assay and the Jerne hemolytic plaque are designed to measure numbers of B cells producing antibody, rather than the total quantity of antibody produced per cell, it is not possible to speculate on the possibility that thermal injury may be associated with a reduction in total quantity of antibody per cell. Strong evidence exists, however, that many of the steps necessary for in vivo activation and proliferation of B

cells may be defective after thermal injury. The defects, including impaired phagocytosis,[6, 30] macrophage dysfunction,[26, 31] impaired antigen presentation,[5] reduced T-cell proliferation and IL-2 production,[9, 10, 32] and the presence of an immunosuppressive serum factor,[7, 33, 34] have all been shown to be characteristic features of thermal injury and may act singly or in unison to impair in vivo production of antibody.

This study shows a clear shift from IgM to IgG antibody production, which may be secondary to increased PGE_2 production that is characteristic of thermal injury. Increased numbers of antigen-specific IgG B cells in burned animals did not, however, translate into increased serum titers of specific antibody, which were persistently lower than sham-burned controls. The data clearly show a profound and long-lasting impairment of IgG antibody responses to T-cell-dependent antigens in thermally injured animals and emphasize the danger of examining in isolation, in vitro parameters of immune function, which may not accurately reflect the in vivo response to antigenic challenge.

REFERENCES

1. Shirani P, Pruitt BA Jr, Mason AD Jr. The influence of inhalational injury and pneumonia on burn mortality. Ann Surg 1987;205:82-7.
2. Curreri PW, Luterman A, Braun DW Jr, Shires GT. Burn injury. Analysis of survival and hospitalization time for 937 patients. Ann Surg 1980;192:472-8.
3. Rosenthal SR, Hartney JB, Spurrier WA. The "toxin-antitoxin" phenomenon in burned and injured human subjects. JAMA 1960;174:99-107.
4. Alexander JW, Stinnet JD, Ogle CK. Alterations in neutrophil function. In: Ninnemann JL, ed. Immune consequences of thermal injury. Baltimore: Williams and Wilkins, 1981.
5. Kupper TS, Green DR, Durum SK, Baker CC. Defective antigen presentation to a cloned T helper cell clone by macrophages from burned mice can be restored with interleukin-1. SURGERY 1985;98:199-206.
6. Allen RC, Pruitt BA. Humoral-phagocytic axis of immune defense in burn patients. Arch Surg 1982;117:133-40.
7. Wolfe JHN, Saporoschetz I, Young AE, O'Connor NE, Mannick JA. Suppressive serum, suppressor lymphocytes and death from burns. Ann Surg 1981;193:513-20.
8. Wood JJ, O'Mahony JB, Mannick JA, et al. Immunoglobulin production and suppressor T-cells after thermal injury. Surg Forum 1984;35:619-21.
9. Moss NM, Gough DB, Jordan AL, et al. Temporal correlation of impaired immune response after thermal injury with susceptibility to infection in a murine model. SURGERY 1988;104:882-7.
10. Wood JJ, O'Mahony JB, Mannick JA, et al. Abnormalities of antibody production after thermal injury. An association with reduced IL-2 production. Arch Surg 1986;121:108-15.
11. Gadd MA, Hansbrough JF, Field TO, et al. Antibody formation after murine injury. J Surg Res 1988;44:649-57.
12. Baker CC. Immune mechanisms and host resistance in the trauma patient. Yale J Biol Med 1986;59:387-93.
13. Sedgwick JD, Holt PG. A solid-phase immunoenzymatic technique for the enumeration of specific antibody-secreting cells. J Immunol Methods 1983;57:301-9.
14. Furuya T, Kumazawa Y, Takimoto H, et al. Enhancement of immunoglobulin G responses in mice against hepatitis B virus surface antigen, influenza virus hemagglutinin vaccine, and tetanus toxoid by 6-O-acylated muramyl dipeptides. Infect Immun 1989;57:1839-44.
15. Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 1965;2:235-54.
16. Nohr CW, Christou NV, Rode H, et al. In vivo and in vitro humoral immunity in surgical patients. Ann Surg 1984;200:373-80.
17. Ertel W, Faist E, Nestle C, et al. Dynamics of immunoglobulin synthesis after major trauma: influence of recombinant cytokines. Arch Surg 1989;124:1437-42.
18. McRitchie DI, Girott M, Rotstein OD, Teodorczyk-Injeyan JA. Impaired antibody production in blunt trauma: possible role for T-cell dysfunction. Arch Surg 1990;125:91-6.
19. Schorr RM, Erschler WB, Gamelli RL. Immunoglobulin production in burned patients. J Trauma 1984;24:319-22.
20. Alexander JW, Moncrief JA. Alteration of the immune response following severe thermal injury. Arch Surg 1966;93:75-83.
21. Teodorczyk-Injeyan JA, Sparkes BG, Falk RE, Peters WJ. Polyclonal immunoglobulin production in burned patients—kinetics and correlations with T-cell activity. J Trauma 1986;26:834-9.
22. Teodorczyk-Injeyan JA, Sparkes BG, Peters WJ. Regulation of IgM production in thermally injured patients. Burns 1989;15:241-7.
23. Phipps RP, Roper RL, Stein SH. Regulation of B-cell tolerance and triggering by macrophages and lymphoid dendritic cells. Immunol Rev 1990;117:135-58.
24. Roper RL, Conrad DH, Brown D, Warner G, Phipps RP. Prostaglandin E2 promotes IL-4–induced IgE and IgG1 synthesis. J Immunol 1990;145:2644-51.
25. Ohmori H, Hikada M, Takai T. Prostaglandin E_2 as a selective stimulator of antigen-specific IgE response in murine lymphocytes. Eur J Immunol 1990;20:2499-503.
26. Miller-Graziano CL, Fink M, Wu JY, Szabo G, Kodys K. Mechanisms of altered monocyte prostaglandin E_2 production in severely injured patients. Arch Surg 1988;123:293-9.
27. Ninnemann JL, Stockland AE. Participation of prostaglandin E_2 in immunosuppression following thermal injury. J Trauma 1984;24:201-7.
28. Wang BS, Heacock EH, Wu AVO, et al. Generation of suppressor cells in mice after surgical trauma. J Clin Invest 1980;66:200-9.
29. Arturson G, Hogman CF, Johansson SGO, Killander J. Changes in immunoglobulin levels in severely burned patients. Lancet 1969;1:546-8.
30. DiMaio A, DiMaio D, Jacques L. Phagocytosis in experimental burns. J Surg Res 1976;21:437-48.
31. O'Riordain MG, Collins KH, Pilz M, Saporoschetz IB, Mannick JA. Modulation of macrophage hyperactivity improves survival in a burn-sepsis model. Arch Surg 1992;127:152-8.
32. Miller CL, Baker C. Changes in lymphocyte activity after thermal injury: the role of suppressor cells. J Clin Invest 1979;63:202-10.
33. Constantian MB. Association of sepsis with an immunosuppressive polypeptide in the serum of burn patients. Ann Surg 1978;188:209-15.
34. Ninneman JL, Fischer JC, Watchell TL. Thermal injury–associated immunosuppression: occurrence and in vitro blocking effect of postrecovery serum. J Immunol 1979;122:1736-41.